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13. ABSTRACT (Maximum 200 Words)

Androgen ablation (AA) constitutes the most common therapy for the treatment of advanced prostate cancer. While initially effective at reducing tumor burden, most patients recur with androgen insensitive disease. There exists a clear need to augment the clinical efficacy of hormone-based therapies, and immunotherapy of prostate cancer represents a promising approach for achieving such augmentation. Moreover, our data indicate that AA affects the immune system both systemically as well as at the prostate. Castration of mice stimulates B and T lymphopoiesis, thymic and bone marrow hyperplasia. The induction of apoptotic cell death following androgen ablation is accompanied by an inflammatory infiltrate comprised predominantly of activated T cells. This AA induced autoimmune-like response exerts limited anti-tumor activity in a mouse prostate cancel model, but the anti-tumor effect is potentially synergistic with CTLA-4 blockade, which promotes the development of autoreactive T cells. We have used the first year of this proposal to obtain and produce all necessary reagents (genes, tumor cell lines, hybridomas, purified antibodies) to position ourselves for studying the effects of AA on prostate cancer immunity as these effecs might significantly influence the ability of a tumor-bearing host to mount an effective immune response.

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Table of Contents

Introduction

Normal and cancerous prostate cells require male sex hormones (androgens) for growth. Consequently, a common treatment for prostate cancer is androgen ablation (AA) therapy. Although AA constitutes an effective means for significantly reducing tumor burden and inducing a phase of remission of tumor growth in prostate cancer, a majority of treated patients recur with untreatable, androgen insensitive tumors. Consequently, there is a need for the development of new therapies that target prostate cancer cells via mechanisms that do not rely on the hormonal sensitivity of the cells. Yet the effectiveness of AA in reducing initial tumor burden makes its continued use likely, so new therapeutic approaches that provide synergistic anti-tumor effects when combined with AA would be attractive.

Considerable interest has been directed recently toward the development of immunotherapeutic approaches for the treatment of malignancy, including prostate cancer. However, it is well established that sex hormones are potent regulators of the immune response and lymphocyte development. Since immune-based therapies will likely be administered in patients undergoing AA therapy, the loss of androgen effects may influence the nature of the host immune response. In general, androgens suppress the immune response and, conversely, castration of mice increases their ability to reject transplanted tissues. However, little is known about the effects of AA on anti-tumor immunity and whether current treatments used to achieve AA in prostate cancer patients affects their immune system.

Preliminary investigations from our laboratory demonstrate that AA affects the immune system both at the systemic level as well as at the local level of the prostate itself. A key characteristic of AA at the systemic level is a potentiation of production of B and T cells, critical cellular components of the immune system. Locally, AA induces activation of T cells that infiltrate the prostate. Evidence from our laboratory suggests that AA alone may stimulate an immune response against prostate tissue which, when combined with an immunotherapeutic agent that blocks the function of a lymphocyte receptor (CTLA-4), can successfully regress prostate tumors in mice. Thus, the effects of AA on the immune system indicate that it significantly influences the effects of immunotherapy, and that these effects might be positive.

This application proposes studies designed to identify the effects of AA on anti-tumor immunity and to determine the mechanism behind any effects. We will test the hypothesis that AA promotes anti-tumor immunity by augmenting the antigen-presenting functions of dendritic cells. Additionally, we hypothesize that the synergistic anti-tumor effects of AA combined with CTLA-4 blockade result either from increased expansion of local infiltrating T cells or through activation of novel autoreactive T cell clones. Finally, we will determine whether AA exerts similar effects on the immune system of prostate cancer patients. The long-term objective of this project is to understand the effects of AA on anti-tumor immunity in prostate cancer and to exploit those effects for the development of successful immunotherapies for the treatment of prostate cancer.

Body

This progress report describes the activities on the grant proposal in the first year. This project had a rough start at Loyola University Chicago as the original P.I. Dr. Ellis left the scientific arena rather suddenly. The project was taken over on a very short notice by Dr. W. Martin Kast an experienced tumor immunologist. The original clinical P.I. for the clinical part of the proposal also left Loyola University Chicago late 2002 and was replaced by Dr. Robert Flanigan. As both original P.I.'s left the institution with resentment the project was left in a non-optimal shape for the current P.I. to pick up. On top of that the new clinical P.I. encountered medical problems shortly after the start of the project that took him out for 4 months. After his return the current P.I. Dr. Kast had also decided to leave Loyola University Chicago for the University of Southern California. This move took place on September 1, 2003. The second year graduate student who was assigned to this project and who joined the lab late 2002 was trained to do the techniques required for this project including castration of mice but did not want to move to USC and left the lab in May 2003. The postdoc assigned to this project arrived 5 months after the project started due to unexpected visa issues. After her arrival the decision of the P.I. to move to USC affected her as she could not move to USC and left the project in August 2003. At this moment we are in the process of moving the grant from University Chicago to USC. Combined this has resulted in that only a small progress was made in the first year.

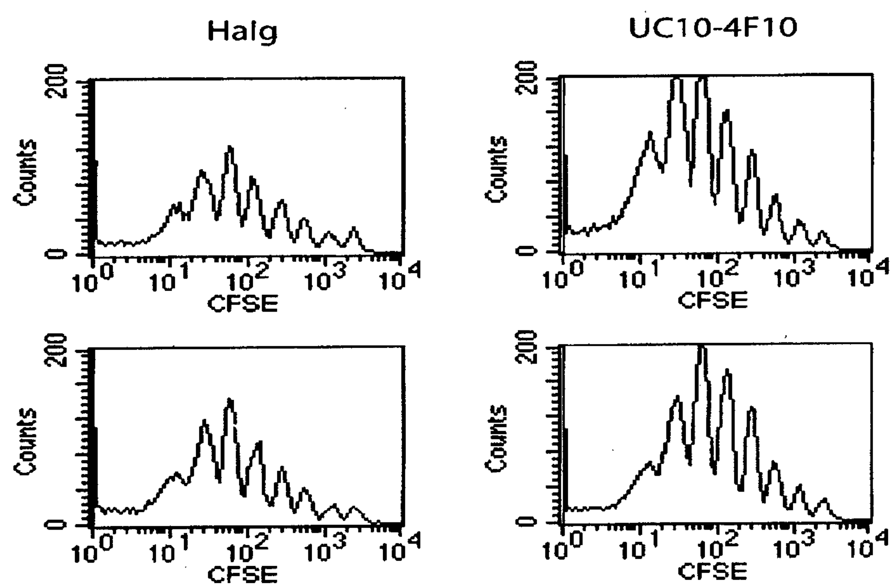
<u>Specific Aim 1</u>: Task 1: Select and grow B7.1+ TC1 and V51 Blim10 tumor cells.

Neither the tumor cells nor the B7.1 gene were left by the original P.I. Therefore new TC1 tumor cells were obtained from Dr. Norm Greenberg and the cDNA encoding murine CD80 (B7.1) was amplified from total C57 Bl 16 spleen RNA by RT-PCR. PCR products were ligated into the pCR II TA cloning vector. Recombinant colonies were sequenced to ensure that no errors were introduced into the cDNA clone during PCR amplification. Once a cDNA clone with the correct DNA sequence was identified the CD80 cDNA was subcloned into the pcDNA III eukaryotic expression vector. Recombinant clones were screened by restriction enzyme digestion and then by DNA sequence analysis to confirm that CD80 was inserted into the vector in the correct orientation. The V51 Blim 10 tumor cell line count not be obtained elsewhere and had to be replaced by another weakly immunogenic tumor cell line. The choice fell on two of them C3 an HPV16 and activated *ras* transformed cell line and B16 a melanoma cell line and both are being transfected with B7.1 by a new postdoc Luz Garcia who started in October, 2003 and who is assigned to this project. She is temporarily paid from start up money until Loyola University Chicago relinquishes the DOD grant.

<u>Specific Aim 2:</u> Task 1: Produce, purify, and characterize anti-CTLA-4 antibody for in vivo experiments.

We have obtained the UC10-4 F 10 anti CTLA-4 hybridoma from Dr. Schoenberger and brought it into culture. This hybridoma was adapted by us to grow under serum free conditions in Gibco PFHM-II medium and grown in roller bottles. After 3 days of culture the supernatant was cleared of cells and concentrated 50 fold using a artificial dialysis kidney. The concentrated supernatant was then dialyzed extensively against PBS, filter sterilized, quantitated by protein assay and store at 4°C. We have now made a batch of 20 mg of purified antibody for in vivo use. The antibody was tested for in vivo activity and demonstrated to enhance the primary expansion of transgenic CTL in vivo. OT-1 CD8+ T cells were labeled with CFSE prior to i.v. injection into B6 mice. Twenty-four hours later the mice were challenged with splenocytes pulsed with ovalbumin. One group was treated with 100µg anti-CTLA-4 antibody (UC10-4F10) or a control antibody (hamster IgG). Three days after challenge spleen and lymph node cells were harvested and analyzed by flow cytometry to monitor OT-I proliferation by CFSE dilution. Figure 1 shows that, although the number of divisions is identical for spleen cells (top row) and lymph node cells (bottom row) in both cases, the magnitude of OT-I expansion is greater in mice treated with the anti-CTLA4 antibody compared to the control group.

Figure 1:



This demonstrates that our purified anti-CTLA-4 antibody is functional in vivo which completes this task.

Specific Aim 3: This aim was scheduled to start in the second year of the project. To prepare for that a new clinical P.I. was sought and found at USC. Eila Skinner, MD, an associate professor of Urology (see attached NIH CV) has agreed to lead the clinical component of this proposal. She will submit the clinical proposal to the USC IRB for their comments and subsequently to the DOD.

Body (continued)

The relative downtime for this project was used to thoroughly analyze the literature on prostate cancer immunotherapy and tumor vaccine developments. This analysis was published in two major reviews Le Poole IC, Bommiasamy Hemamalini and Kast WM. Recent progress in tumour vaccine development, Expert Opin. Investig. Drugs 12(6):971-981, 2003 and Markiewicz MA and Kast WM Advances in Immunotherapy for Prostate Cancer, Advances in Cancer Research 0065-230X/03 that mentioned the support of this grant. Despite the set back at the start of this project due to the listed problems that arose on the positive side is that not much of the awarded grant was spent and that the project is now much better situated for success in a new institution, with a newly appointed postdoc, with all necessary reagents in place and with a newly appointed clinical PI. A no-cost extension year later on will allow us to catch up and complete our tasks.

Key Research Accomplishments

1. Cloned the murine B7.1 gene.
2. Produced, purified and characterized a large batch of anti-CTLA-4 antibody for in vivo experiments

Reportable Outcomes

Manuscripts, abstracts, presentations:

Two articles

1. Le Poole IC, Bommiasamy Hemamalini and Kast WM. Recent progress in tumour vaccine development.
2. Markiewicz MA and Kast WM. Advances in Immunotherapy for Prostate Cancer.

Patents and licenses applied for and/or issued:

None

Degrees obtained that are supported by this award:

None

Development of cell lines, tissue or serum repositories:

None

Informatics such as databases and animal models, etc:

None

Funding applied for based on work supported by this award:

None

Employment or research opportunities applied for and/or received on experiences/training supported by this award:

None

Conclusions

We are attempting to understand how androgen influence the immune system. As androgen ablation is an often executed treatment in advanced prostate cancer patients such knowledge is important for treatments that activate the immune system. This newly awarded grant has had some start-up problems but is now poised for successful completion. Important reagents like the B7.1 gene, the tumor cell lines TC1, C3 and B16 and the hybridoma UC10-4F10) were obtained, a large batch of purified anti-CTLA-4 antibodies was produced and a new infrastructure was build to execute the remainder of the tasks.

References

Le Poole IC, Bommiasamy Hemamalini and Kast WM. Recent progress in tumour vaccine development, Expert Opin. Investig. Drugs 12:971-981, 2003.

Markiewicz MA and Kast WM. Advances in Immunotherapy for Prostate Cancer, Advances in Cancer Research 87:159-194, 2003.

Appendices

Le Poole IC, Bommiasamy Hemamalini and Kast WM. Recent progress in tumour vaccine development, Expert Opin. Investig. Drugs 12:971-981, 2003.

Markiewicz MA and Kast WM. Advances in Immunotherapy for Prostate Cancer, Advances in Cancer Research 87:159-194, 2003.

NIH CV of Eila Skinner, M.D. as clinical co-investigator for the project.

APPENDIX 1

Expert Opinion

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Monthly Focus: Oncologic

Recent progress in tumour vaccine development

I Caroline Le Poole[1,2†], Hemamalini Bommiasamy[1] & W Martin Kast[1,3]
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Immunotherapy offers an exciting opportunity to treat human cancer. Analysis of tumour-associated antigens is progressing. Assisted by animal models, such knowledge can be used to design tumour vaccines. By including adjuvants to increase immunogenicity, several tumours previously thought to be non-immunogenic are now considered targets for tumour vaccines. Newly acquired knowledge regarding dendritic cell physiology is incorporated in newly designed vaccines that are currently in Phase I and II trials. Such assessment provides the overall conclusion that tumour vaccines are safe and deserve a more prominent place in the sequel of treatments for human cancer.

Keywords: adjuvants, antibodies, antigens, dendritic cells, immune monitoring, immunotherapy, T cells, tumour vaccine

Expert Opin. Investig. Drugs (2003) 12(6):971-981

1. Introduction

Metastasis of tumour cells presents a major challenge for the development of effective treatment modalities for cancer. It prevents cancer from being effectively cured by surgical procedures and provides the tumour with hiding places that cannot be reached by common treatment modalities. The immune system can provide a solution to this problem. Its specificity leaves surrounding tissues unharmed and its plasticity helps it find residual tumour cells. Herein lies the crux of cancer vaccine research: how to enable the host immune system to kill tumour cells, leaving normal tissue cells unharmed. Developments in tumour vaccine research in the year 2002 and developments expected in the near future are described here.

An essential part of vaccine development lies in the identification of tumour-associated antigens. Investigating the immunome is providing a host of novel antigens for tumours previously considered non-immunogenic [1]. Where antigens have not yet been identified, tumour cells or parts thereof can evoke antitumour immunity particularly when used to pulse dendritic cells (DCs). In this regard, the vastly increasing knowledge of DC physiology has been very supportive. Efficient *in vitro* cultivation, pulsing, maturation and administration procedures render DCs effective front-line soldiers in the antitumour response.

Whereas cytotoxic T cells have long been considered the focus point of tumour immunology, the development of tumour-specific humoral responses is also gaining momentum. Advantages to antibodies include the accessibility of the tumour cell membrane, which can often be distinguished from that of neighbouring cells with normal physiology. In particular, the carbohydrate moieties expressed on the tumour cell membrane expose the malignancy. Moreover, such recognition is not dependent on sustained expression of human leukocyte antigen (HLA) molecules. In either case, T helper (T_H) cells are involved in elicitation of antitumour immunity. Such T cell help need not necessarily be tumour specific, although activation of tumour-specific T_H cells can provide lasting memory responses essential to prevent tumour recurrences. The cytokine profiles of T_H cells can skew the immune response

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towards cellular immunity (type 1 cytokines) or humoral immunity (type 2 cytokines). This very fact provides tumour immunologists with a means to manipulate vaccine efficacy by including cytokine treatment to skew the response in favour of the most effective arm of the immune response for a particular antigen.

Mere induction of cytotoxic T-cell responses is generally insufficient to provide a cure for existing tumours. The escape mechanisms put in place within the tumour environment can prevent T cells from recognising and killing the tumour. Besides assistance from antibodies, support is also drawn from adjuvant therapy to improve expression of costimulatory molecules and cytokines involved in enhancing T-cell responsiveness. Moreover, selection and cloning protocols for T-cell receptors (TCRs) will enable the development of off-the-shelf reagents enabling induction of high affinity responses.

Many Phase I/II trials have shown that tumour vaccines are safe. Since these trials are generally performed in immune compromised, terminally ill patients, it is not surprising that antitumour responses have seemed disappointing. By contrast, animal models continue to provide support for the notion that tumour vaccines can be effective not only in a prophylactic but also in a therapeutic setting. It will be important to move immunotherapeutic strategies forward in the sequel of treatment options for human malignancies in order to use this strategy to its full potential [2].

2. Tumour antigens

Whole tumour cells are used as vaccines targeting immunogenic tumours where individual antigens have not yet been identified, for example, in renal cell carcinoma [3]. Unfortunately, these crude preparations include immune suppressants [4]. Thus, particularly for less immunogenic tumours, it is important to specify individual tumour-associated antigens. Surprisingly, the majority of antigens identified to date are self-antigens also expressed in the normal counterpart of malignant tumour cells. The immunogenic potential of such differentiation-associated antigens was recently reported for C98 in human gastric ring cell carcinoma [5], CD20 in B cell lymphoma [6], parathyroid hormone-related protein (PTH-rP) in epithelial cancers [7] and cleavage and polyadenylation specificity factor (CPSF) for colon cancer [8]. Although the application of such vaccines is not necessarily associated with responses to normal tissue cells, there is a lingering concern for autoimmunity. Ideally, vaccines will contain antigens expressed almost exclusively within tumours. Testes-associated antigens are frequently expressed in malignant settings and attempts have been made to identify humoral target proteins by screening a testes cDNA expression library with patient serum [9]. Such cancer/testes antigens include NY-ESO, HER2 and MAGE antigens. Since expression of cancer/testes antigens is generally associated with malignant transformation of cells independent of their lineage, such antigens can find applications for multiple tumours.

To further improve such applications, peptides binding to the groove of major histocompatibility complex (MHC) molecules are predicted by computer algorithms [10]. Alternatively, peptides can be altered for improved HLA binding as demonstrated for telomerase reverse transcriptase (TERT), p53 and carcino-embryonic antigen (CEA) [11-13]. These predictions do not take into account how proteins are naturally processed to be presented at the cell surface. Frequently, predicted immunogenic peptides are not represented at the cell surface of actual tumour cells. Obviously such peptides should not be included in tumour vaccines as the cryptic epitopes will compete with naturally processed peptides for recruitment of tumour-reactive T cells [14]. As an example, among predicted HLA-A2-binding peptides from prostate-specific membrane antigen (PSMA), five induced CTL but only one induced T cells reactive with prostate tumour cells [15]. In the case of NY-ESO, peptide 157-167 displays better binding to HLA-A2 and elicits high avidity T cells that are not reactive to the tumour in contrast to peptide 157-165 [16,17]. Whereas these findings support the use of minimal peptides, others have reported multivalent peptide representing multiple antigens or simultaneously stimulating CD4 and CD8 responses [18-20]. Full proteins have also been modified to exclude regions potentially interfering with an immune response, as reported for HER2 [21,22]. Although antigens unrelated to the tumour have been promoted to provide T cell help, the added value of using tumour-specific class-II-binding peptides may lie in the improved induction of memory responses to the tumour, generating a vaccine with longer lasting efficacy. Whatever the immunogen of choice may be, the means by which it is introduced to the immune system can greatly affect the potency of a resulting vaccine as discussed below. This is clear from the relatively limited efficacy of ~ 20% reported for peptide vaccines [23,24].

3. Vaccine preparation and delivery

Tumour antigens can be used to boost host immune responses to the tumours from which they are derived. Resulting vaccines may consist of tumour cells or cell lysates, tumour-associated proteins or peptides, naked RNA or DNA versus recombinant viral vectors or bacteria encoding the tumour antigens. Major differences in vaccine efficacy have emerged among them. A recent example is found in mucin-1 (MUC-1) core protein with IL-2 expressed by bacillus Calmette–Guerin (BCG), which appears to be more efficacious than mature MUC-1 as assessed in peripheral blood lymphocytes (PBL)-reconstituted severe combined immunodeficient (SCID) mice [25].

Tumour cells frequently display an undifferentiated phenotype, leaving few specific antigens to be targeted by the immune response. Such tumour cells can be differentiated or matured *in vitro* by exposing them to cytokines or transfecting costimulatory molecules or cytokine genes to form potent vaccines for acute lymphoblastic leukaemia (ALL) and acute myeloid leukaemia (AML) [26]. Besides autologous tumour cells, allogeneic tumour cell lines can form a well-defined and readily available source of antigens [27]. Such tumour cells can also be mixed with normal tissue cells (i.e., fibroblasts) transfected to express cytokines. Interestingly, this does not lead to immune responses directed against the tissue cells [27]. Dead tumour cells contribute to the antigenicity of vaccines, as shown for autologous tumour cells in melanoma [28]. Cells can also be induced to form syncytia by transgenic expression of vesicular stomatitis virus G glycoprotein (VSVG) for enhanced immunogenicity [29].

An exciting development is the introduction of tumour antigens into highly immunogenic host cell lines. The efficacy of this strategy was shown by enzyme-linked immunospot (ELISPOT) analysis following gp100 expression in IL-2-secreting cells [30]. Where individual proteins are used for vaccination, crosslinking can enhance the efficacy as shown for idiotypic proteins used to treat B cell malignancies [31]. Generating tubular structures with immunogenic peptides has a similar effect [32]. This is possibly explained by the different receptor molecules expressed by recipient DCs used to bind and engulf antigens of different formats [33].

Whether antigens are introduced as proteins, RNA or DNA, the central cell type targeted by vaccines is the DC. This realisation has led to a goldrush in research to understand the biology of this cell type, from its origin to its maturation and migration onto its presentation of antigen in organ-draining lymph nodes to elicit immune responses to tumour-associated antigens.

4. Dendritic cells and tumour immunity

DCs have become an integral part of tumour vaccines. Factors affecting the perceived therapeutic potential of DCs include the means of recruiting DCs, methods used for target antigen loading, modes of DC maturation, the routes used to administer the cells and finally the immune monitoring methods used [34]. This can be extremely important for the outcome of DC-mediated vaccines as there is a fine balance between immune tolerance and immune activation determined mainly by DC physiology [35].

Plasmacytoid DC numbers can be enhanced by Flt3L. Introduced in the form of naked DNA in combination with MUC-1 peptide, this has led to the recruitment of functional DCs [36]. Efficient propagation of DCs from monocyte precursors in the presence of polycytidylic acid (poly I:C) within 24 h was reported [37].

DCs fused with leukaemic cells, pulsed with tumour lysates (necrotic cells) or pulsed with apoptotic cells proved less effective, respectively, for AML [38]. This is in agreement with consensus opinion that necrotic cells are more immunogenic than apoptotic cells. In renal cell carcinoma, DCs pulsed with apoptotic cells proved more immunogenic than necrotic cells [39]. Such differences may be mediated in part by expression of heat shock protein-60 (HSP60) and HSP72 on the cell surface of apoptotic cells, which can stimulate DCs and induce specific cytotoxic T cells [40]. Since there are so many factors affecting the efficacy of uptake and maturation of DCs, it is most likely that a comparison of methods would always fall short on that issue. It is also possible that such comparisons overlook the incorporation of a population of myeloid suppressor cells, expressing granulocyte and monocyte markers and inhibiting T-cell survival, as identified in mouse models of glioma [41].

Cell fusion has been assessed for immature versus mature DCs and it was clearly shown that the latter deliver the better option [42]. Unfortunately, at 3 – 18% efficiency, cell fusion appears to be a very inefficient process by current methods [43].

Uptake of antigens can be improved by making use of Fc receptors expressed on the DC cell surface. Using immune complexes of model antigen ovalbumin (OVA) and antibodies, it was shown that such immune complexes efficiently induce T-cell-mediated responses to OVA [44]. Interestingly, these studies would support the notion that humoral responses ultimately support antigen-specific T-cell responses as well [44]. However, among Fc receptors, stimulation of Fcγ receptor IIB (FcγRIIB) may deliver tolerising signals. Thus, inhibiting this receptor on the DC cell surface is likely to enhance antitumour reactivity [45]. Other factors affecting uptake of tumour antigens are granulocyte-macrophage colony-stimulating factor (GM-CSF), particularly in its membrane bound form [46,47], and DC-specific intercellular adhesion molecule (ICAM)3-grabbing non-integrin (DC-SIGN), important for uptake of HIV [48].

Transgenes introduced into DCs are more effective in mature versus immature DCs [49]. Such transgenes may include IL-18, stimulating Type 1 immune reactivity as well as epitope spreading [50]. DC maturation signals include β-defensins bound to DCs via Toll-like receptor-4 (TLR-4), which induces a Type 1 immune response [51]. DC maturation promotes cell survival, involving upregulation of Bcl-X (l) and Bcl-2 [52].

The cytokines secreted by DCs guide the type of immune response that follows. An example is found in IL-18 secretion supporting Type 1 reactivity and cell-mediated immunity to tumours. By contrast, reduced IL-12 secretion by DCs stimulates a Type 2-mediated response guiding secretion of antibodies by B cells [53]. Antibodies were long thought to be less important in immune reactivity to tumours, mainly because their range is limited to the membrane of the target cell. However, several tumour types are well suited for a humoral attack and this realisation has reignited an interest in antibody-mediated antitumour responses as discussed below.

5. Antibody-mediated immunotherapy

B cells express antibodies on their cell surface and clonal B cell lymphomas will carry a unique idiotype that can be targeted without affecting viability or efficacy of other B cells. Resulting vaccines are among the first to be tested in Phase I/II clinical trials. Resulting toxicity is low and responses are modest

[54,55]. A disadvantage to idiotype-based vaccines is that each patient requires development of an individual vaccine. The task of developing patient-restricted vaccines is made easier by reverse transcription-polymerase chain reaction (RT-PCR) followed by cloning the product into an expression vector as tested in mice [56]. Another DNA vaccine was constructed by fusing the single chain Fv to a tetanus toxoid sequence, similarly resulting in induction of Type 2 cytokine responses [57]. Others have focused on developing a universal B-cell vaccine, targeting CD20 peptide linked to a foreign IgG.Fc fragment and eliciting a humoral response that reduced the number of available B cells in mice [58]. Anti-idiotype vaccines can also be applied towards shared rather than individualised membrane antigens. In this respect, TriGem™, an anti-idiotype vaccine to three gangliosides in melanoma demonstrated clinical activity in a Phase II trial [59].

Membrane antigens appear to elicit a primarily humoral response. Apart from the idiotypes vaccines mentioned above, examples of membrane antigens include PSMA as shown in mice [60], KH-1 in adenocarcinoma [61] and ganglioside GM3, also tested as an anti-idiotype vaccine, for melanoma [62]. Interestingly, the efficacy of KH-1 carbohydrate antigen vaccines as shown by IgG versus IgM responses is dependent on the mode of linkage to the carrier protein [62].

Passive immunisation can also be applied to membrane antigens, where suitable antibodies are available. Well characterised mAbs of mouse origin are humanised to eliminate anti-Fc responses that will otherwise neutralise the vaccine. An example is found in trastuzumab humanised antibodies to HER2 as considered for breast cancer [63].

Activation of both DCs and T cells is accelerated by adjuvant therapy, particularly in the form of cytokine administration and introduction of costimulatory molecules. Ongoing research investigating the efficacy of such adjuvant therapy is discussed below.

6. Adjuvant therapy

The term 'adjuvant therapy' is used here to refer to therapeutic measures that support and enhance the efficacy of existing tumour vaccines. In this respect, cytokine-modified tumour vaccines can avoid tolerisation [64]. Indirectly, adjuvants stimulating Type 1 cytokine secretion can improve vaccine efficacy through enhanced IFN-γ expression [66]. Thalomide analogue ACTIMID is an immunomodulatory drug in use to do just that: enhancing antitumour activity by stimulating Type 1 cytokine secretion [66]. Cytokine expression can also be introduced by transduced tumour cells, as shown for glioma and gliosarcoma treatment [67]. The most important cytokine to include in such treatment appears to be GM-CSF. In fact, GM-CSF proved more helpful than IL-12 for glioma treatment and further improvement was noted for tumour cells also expressing B7-2 [68]. The same holds true for hepatic cancer, where RANTES (regulated on activation, normal T-cell expressed and secreted) is introduced in addition to GM-CSF and B7 [69].

GM-CSF and IL-2 can improve the efficacy of existing vaccines strategies as shown for idiotype vaccines for B-cell lymphomas [70] as well as for CEA-TRICOM administration, where costimulation is also provided by B7-1, ICAM-1 and leukocyte function-associated antigen-3 (LFA-3) [71]. Importantly, such treatment has demonstrated anticancer efficacy in the absence of autoimmune responses [72].

IFN-α in combination with IL-2 may be helpful for renal cell carcinoma [73]. Attempts to reduce IFN-α toxicity during melanoma treatment by lowering the dose have resulted in reduced efficacy [74]. Shorter high-dose treatment has been proposed to treat patients at intermediate risk for tumour recurrences, for which no other treatment is currently available [74].

HSP110 is new on the immunotherapy scene and was shown to enhance CD8 and natural killer (NK) responses [75]. HSP70 has shown merits for liver cancer, where tumour cell-derived HSP70 fractions proved to be more effective than normal liver cell-derived HSP70 fractions [76]. This is explained by the fact that HSPs chaperone antigenic peptides representative of the cell type from which they are derived. Such antigens are more efficiently processed and presented by DCs. Similarly, poly-L-arginine can greatly enhance peptide delivery to cells and thereby enhance the efficacy of peptide vaccines [77,78].

CD40L is a costimulatory molecule that, when expressed in B-cell lymphoma cells, is protective in animal models [79].

Much of the initial testing for tumour vaccines is carried out in mice. Obviously, it is important that the animal model accurately represents human cancer and its response to treatment. In mice, the contribution of different cell types or molecules can be accurately investigated by depleting them either by using antibodies or by employing knockout mice completely lacking the molecule of interest. This line of research is revealing some important and particularly unexpected aspects of tumour vaccines.

7. Mouse models

Mouse models are being generated where an oncogene is expressed under a tissue-specific promoter. First used to generate mice spontaneously developing melanoma, current applications include a model for breast cancer. In the latter case, mice transgenic for polyomavirus middle T were also transgenic for MUC-1, so that MUC-based vaccines could be tested in this model expressing MUC-1 as a self-antigen [80]. It was shown that prophylactic vaccines consisting of tumour cell-fused DCs offer protection in 60% of mice [80]. The same MUC-1 gene is also expressed in a mouse model for pancreatic cancer. In the resulting MET mouse model, treatment with staphylococcal enterotoxin followed by DCs fused with tumour cells resulted in improved survival which correlated with MUC-1 expression [81].

As many vaccines are generated with HLA-A2$^+$ patients in mind, HLA-A2 transgenic mice are proving helpful to test such vaccines. In this regard, a tumour cell line representative of cervical cancer in humans was generated by introducing activated *ras* together with HPV16 E6 and E7 genes into mouse fibroblasts. This tumour model has proven useful in testing the efficacy of several types of vaccines for the treatment of cervical cancer [82]. Meanwhile, improvements were made to immune monitoring techniques specifically for HLA-A2 transgenic mice by generating HLA-A2/H2K(b)A tetramers to improve sensitivity of the screening by allowing for mouse CD8 binding [83]. It is important to bear in mind that codon usage in mice differs slightly from humans. Others have proposed to compensate for this by restoring mouse preferred sequences for the immunogenic gene product, with some success [84].

The opposite approach involves depleting cells or molecules of potential importance for vaccine efficacy. For example, it was shown that successful treatment with cyclophosphamide, hapten-dinitrophenyl (DNP)-treated tumour cells and BCG for mammary tumours was dependent on both CD8 and CD4. Also, cytokines IFN-γ and TNF contributed to the efficacy of the treatment [85].

Alternatively, genetically-deficient mice can be reconstituted with a component of interest as shown for lymphopenic recombinant activating gene-1 (RAG1) mice reconstituted with naive T cells and vaccinated with melanoma vaccine. T_H1 and cytotoxic T cell-1 (T_C1) responses develop in the mice in response to treatment [86].

These results confirm the notion that mouse models provide us with valuable information regarding antitumour immune responses. Also, the fact that tumour vaccines frequently prove effective in mice may be a reflection of the fact that vaccines can be used to successfully treat tumour patients when used in a controlled and timely fashion.

Moreover, human trials currently ongoing or recently completed have consistently shown tumour vaccines to be a safe option with few side effects. Some recent studies are highlighted below.

8. Vaccine trials

Recent vaccine trials are mostly Phase I and II trials, primarily focused on safety and only marginally on the efficacy of the vaccines. Of all trials published recently, approximately half apply to melanoma, underscoring the relative amount of attention this deadly form of cancer has received within the field of tumour vaccines. Such attention is justified by the poor cure rate for melanoma by conventional treatment of only 10% [87]. Moreover, melanoma tumour cells are relatively immunogenic as a consequence of differentiation antigens expressed in the melanosomal compartment of the cell. Newly published trials include treatment of stage II patients with gp100 and tyrosinase-derived peptides. Efficacy of the vaccine as assessed by immune monitoring revealed immune responses in 80% of patients, including epitope spreading to the melanoma antigen recognised by T cells (MART) [88]. Increased ELISPOT numbers were also found in a study of 42 metastatic melanoma patients treated with HSP96-peptide complexes. Two complete responders were reported [89]. When loaded onto DCs, MAGE-derived peptides mounted T_H1 responses in the majority of patients [90]. Tyrosinase peptide therapy also proved helpful in preventing recurrences in two frequently relapsing patients [91].

Besides peptide vaccines, allogeneic tumour cell line homogenates have been assessed in large trials. In resected stage IV melanoma patients, a 19% improvement in 5 year survival was noted using Canvaxin™ [92]. The same vaccine provided a > 100% increase in overall survival for stage II melanoma, supporting the notion that tumour vaccines are more successful when provided to earlier stage patients [93]. However, an increase in overall survival from 88 to 152 months was considered nonsignificant for patients treated with vaccinia melanoma lysates [94]. Further improvement may be possible by combining such allogeneic tumour therapy with IFN-α treatment or other adjuvants [95]. Survival appears to correlate with antibodies to the immunogen in stage II patients with excised tumours [96]. Less characterised autologous tumour vaccines may be useful to prevent recurrences [97].

Clinical trials for other tumour-specific vaccines include recombinant vaccinia virus expressing human prostate-specific antigen (rV-PSA) for prostate cancer [98], CEA-hepatitis B surface antigen [99], human chorionic gonadotropin (HCG)-derived CTP37 conjugated to diphtheria toxin and recombinant canarypoxvirus encoded p53 for colorectal cancer [100], and Her2neu peptide 369-377 for breast and ovarian cancer [101]. Adjuvant therapy in the form of B7-1 transfected into renal carcinoma cells provided responses in 4 out of 15 patients [102].

9. Monitoring

Responses to tumour vaccines correlated with delayed type hypersensitivity (DTH) responses in stage III and IV melanoma patients [103], but a different study concluded that DTH responses are not an accurate measure of the ability of cancer patients to mount an immune response [104]. This apparent discrepancy is possibly explained by immune escape mechanisms suppressing effective immunity in the latter case. Since no single parameter correlates fully with responsiveness to vaccines, measures of humoral and cellular responses are best analysed in addition to clinical parameters for accurate monitoring of vaccine efficacy. To optimise the composition of tumour vaccines, it can be important to fully define the immune response that follows. This is illustrated by reports of the response to peptides modified for optimal binding to MHC. Such vaccines can elicit stronger cytotoxic T lymphocyte (CTL) responses, but these T cells are not necessarily reactive to tumour cells [105]. Quantitative analysis of responder T cells as well as functional assays are needed to draw the conclusion that such peptides are not naturally generated within tumour cells and may impede a productive immune response. Cytokine secretion by responder T cells is considered a measure of potential vaccine efficacy; in fact, ELISPOT assays are considered the premier monitoring measure for tumour vaccines [106]. Quantitative RT-PCR is gaining momentum as a more sensitive method to define cytokine gene expression by responder T cells [107]. Similarly, double staining by tetramers and functional markers such as perforin and CD45RA/CD27 is proving useful in defining vaccine potential [108]. Meanwhile, analysis of responsive T cells is extended from defining the antigenic peptide for a particular T-cell clone to the analysis of TCR-Vβ rearrangement and junctional diversity of T cells infiltrating tumours [109]. It was observed that adjuvant treatment by DNP treatment preferentially induced specific T-cell clones [109].

10. Expert opinion and future developments

Novel tumour antigens for less immunogenic tumours can be predicted by microarray analysis or by the use of patient sera to screen protein arrays as assessed for lymphoma [9]. Once potential tumour antigens are identified, derivative peptides binding to MHC class I molecules can be predicted by computer algorithms. It will be very helpful if properties of gene products can be defined that determine the proteasomal cleavage sites. Such knowledge can be used to face the challenging task of developing computer algorithms that can more accurately predict naturally processed peptides. When combined with programs predicting preferential MHC-binding peptides, an accurate prediction can likely be made of peptides of potential use in tumour vaccines. As MHC class II presented tumour antigens are clearly important for the induction of a memory response to a tumour, computer algorithms have also been put in place to accurately predict peptides of use for eliciting an antitumour response.

Where T cells have proven to be of high affinity for a tumour antigen, the receptor specificity can be cloned and introduced into activated T cells of a different specificity, thereby generating bispecific and active T cells that can respond to tumour cells [110]. Similarly, since humoral responses are more effective than previously assumed, passive use of the bispecific antibodies can improve the affinity of existing antibodies to tumour cells. It may also be possible to enhance the efficacy of bispecific T cells by co-transfecting costimulatory molecule genes or cytokine genes.

Validation of animal models is improving by generating reagents suitable for monitoring of transgenic mice, such as the tetramers that take into account that the species-specific α3 domain of the TCR is important for recognition by CD8 molecules [83]. In general, the immune response observed in the mouse can be somewhat different than is expected in humans due to subtle difference in codon usage [42]. Where this proves important, codon adaptations may be introduced to overcome such a limitation. Knockout mice and antibody-depleted mice are proving increasingly useful to define the role of individual gene products in tumour vaccine efficacy. Examples include the revelation that IFN-γ is essential for responses to MUC-1 in mice, whereas IL-12, IL-10 or IL-4 are not [111]. It will be important to define whether essential versus optional cytokines differ among responses to different tumours.

Most progress is perhaps to be expected within the field of DCs. It has become apparent that different DC subsets exist (i.e., myeloid, lymphoid and plasmacytoid DCs) sharing an important role in antigen presentation. At this point, it appears that the different subsets regulate different parts of the immune response and respond to different activation and maturation signals, a process that is dependent primarily on their expression pattern of TLRs [33]. Development of different subsets of DCs can be affected by the cytokines that precursor cells are exposed to. In turn, the different subsets generate different immune-activating cytokines which can influence the type of immune response that develops. It follows that the efficacy of a DC-based tumour vaccine can be affected by its record of cytokine exposure.

The efficacy of tumour vaccines is also clearly affected by the route of administration. It has been shown that optimal efficacy of a tumour vaccine was obtained by intranodal delivery [112]. Such knowledge is important in justifying vaccine trials making use of optimised routes of administration. Comparative efficacy of tumour vaccines is yet to be investigated for different vaccines. For example, the optimal injection site for DC-based vaccines may be dependent on the extent of maturation that DCs have undergone *in vitro* since immature DCs appear to introduce tolerance rather than immune activation. When vaccinating with tumour antigens, optimal sites may depend on cytokine status of the tissue and the extent of antigen processing required prior to presentation.

Vaccine trials executed to date have been concerned mainly with the safety of vaccines. Since such trials are generally executed in immune compromised end-stage patients, it follows that the efficacy of vaccines included in such trials has not been tested to its full potential. Consequently, much more is to be expected when vaccines are applied in earlier stages of the disease process. Later stage trials are underway that can support this notion. Further support is to be expected from prophylactic vaccines. A major success with such vaccines was obtained for vaccines that potentially prevent the development of cervical cancer [113]. Such prophylactic vaccines can prove particularly successful where tumour antigens have been identified that are not expressed in the normal counterpart of the tumour cells, so as to avoid a risk for the development of autoimmune phenomena.

Immune monitoring strategies have much to gain from combined assessment of multiple parameters. Techniques are underway that can quantify tumour-specific T cells (e.g., by tetramer analysis) and simultaneously assess their state of activation (e.g., by assessment staining for intracellular cytokines or apoptosis-inducing molecule). Since antibody-based vaccines are making their mark in the field of tumour vaccines, it is becoming increasingly important to include an assessment of antibody-mediated parameters when defining immune responses to a vaccine. Since awareness has risen that the type of DC presenting a tumour antigen can affect immune reactivity to a given antigen, monitoring trials are likely to include more parameters to assess TLR expression profiles on recruited DCs. Interestingly, DCs were shown to possess cytotoxic activity towards malignantly transformed cells by means of membrane expression of TNF family members [114]. Thus, monitoring trials may come to include an assessment of expression of such molecules on the DC cell surface.

A recent study demonstrating functional activity, proliferation and trafficking to the tumour sites of T cells adoptively transferred to melanoma patients demonstrated that tumour vaccines can be effective [115]. Simultaneous development of immune responsiveness to pigment cells supports the notion that immunotherapy can be associated with autoimmune responses. Differentiating between vaccines that do or do not elicit autoimmune responses constitutes an immediate challenge for vaccine researchers.

In general, many developments are underway to further improve the composition and application of tumour vaccines. Tumour vaccines are among the most exciting developments in the war against cancer. Most of our knowledge has been acquired with respect to immunogenic melanoma tumours but the field is expanding and other tumours are included among potential candidates for vaccine treatment. As the application of vaccines can be moved forward in the patient treatment schedule, vaccinology will definitely gather further momentum and find broader applicability to include tumours that are considered less immunogenic at this time.

11. Conclusion

Tumour vaccines appear to be safe and effective when used in a prophylactic setting or when used to prevent tumour recurrences after surgical tumour resection. The current challenge for vaccine research is to elicit responses that can shrink existing tumours. Adjuvant therapy and a better understanding of DC physiology are contributing to the success of such tumour vaccines. When applied to tumour patients in earlier phases of their disease, tumour vaccines can make a major contribution to tumour shrinkage.

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Advances in Immunotherapy for Prostate Cancer

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Prostate cancer is the most common malignancy in American men. Metastatic prostate cancer is incurable, with the currently best treatment, androgen ablation, being only palliative. Therefore, there is a need to develop new, more effective therapies against this disease. Multiple immunotherapeutic strategies are being explored for the treatment of prostate cancer, with the hope that such treatment will be more effective and have fewer side effects than current treatment options. Several immunotherapy strategies have been shown to be effective against prostate tumors in animal models, and many of these strategies are beginning to be tested in clinical trials for their efficacy against human prostate cancer. It is likely that effective treatment of prostate cancer will require the use of both immunotherapeutic and traditional approaches in multimodality treatments. In addition, for immunotherapy to be effective against prostate cancer, ways to overcome immune evasion and immunosuppression by the tumor cells will need to be developed.

I. INTRODUCTION

Prostate cancer is a major public health problem in the Western world, as exemplified by the fact that it is the second leading cause of death due to cancer in men in the United States (Tjoa and Murphy, 2000). Conventional treatment of early, localized prostate cancer, such as radiation therapy and

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radical prostatectomy, often fails to rid patients of micrometastatic disease and metastases frequently develop. Androgen ablation is effective initially in metastatic disease, but in most cases, androgen-independent tumors develop, and there is currently no effective treatment for metastatic, androgen-independent prostate cancer. Therefore, there is a push to develop better treatments for both early and metastatic prostate cancer, and one treatment modality under exploration for the treatment of prostate cancer is immunotherapy.

Immunological treatment strategies for cancer fall into the two general categories of passive and active therapy. Passive immunotherapy involves direct administration of effector molecules or cells to a patient and requires no involvement of the patient's own immune system. Active immunotherapy approaches attempt to elicit a response from the patient's own immune system. In addition, both passive and active immunotherapy can be nonspecific or specific in nature. Nonspecific immunotherapy aims to induce inflammation or otherwise enhance the immune response that is already present in a patient, whereas specific immunotherapy requires specific antigen recognition by B cells/antibodies and/or T cells. These forms of immunotherapy that are under exploration in prostate cancer are summarized in Table I. As the study of immunologic approaches for prostate cancer treatment is still in the exploration stage, the best indication of possible effectiveness of many treatments has been seen in preclinical animal models. Therefore, both results obtained in these animal models, as well as those acquired in clinical trials, are discussed in this review.

The development of novel prostate cancer treatments has been hampered by a lack of relevant animal tumor models. To date, the majority of animal prostate tumor models have been conducted with transplantable tumor models in which a host is suddenly given the burden of a large tumor mass. Such models include the transfer of rat Dunning prostate tumor cells into syngeneic Copenhagen rats (Isaacs *et al.*, 1978, 1986). Although much has been learned from these models, spontaneous tumor development models better mimic the disease progression in cancer patients and allow for more thorough preclinical testing of therapies. Such a murine model of chronic prostate cancer development has become available and is known as the transgenic adenocarcinoma of the mouse prostate (TRAMP) (Greenberg *et al.*, 1995). TRAMP mice express the SV40 large T antigen (Tag) under the control of the prostate-specific rat probasin promoter, causing males to develop prostate cancer with high penetrance. In addition, Tag expression is androgen driven and developmentally regulated, leading to transient regression of TRAMP tumors following androgen withdrawal with subsequent recurrence. This progression mimics that seen in human prostate cancer, leading to the emergence of fatal androgen-independent prostate cancer. These mice provide a good preclinical animal model for testing possible therapies for prostate cancer, and studies described here using this model deserve special attention.

Table I Current Immunotherapeutic Approaches in Prostate Cancer

Type of immunotherapy	Examples	Selected references
Passive immunotherapy		
Non-specific	Cytokines	1–5*
Specific	mAb	6–17
	T cell adoptive therapy	18–21
Active immunotherapy		
Non-specific	Microbial products	22–27
	Cytokines	28–30
Specific	Enhancement of endogenous T cell activity	31–34
	Improvement of APC function	35–37
	Induction of a tumor-specific T cell response	
	Genetically engineered tumor cell vaccines	38–41
	Whole protein/peptide vaccination	42–43
	Naked DNA vaccination	44–46
	Recombinant viral vaccines	46–50
	DC-based vaccines	51–54
	Immunization with xenoantigen	55
	Induction of a tumor-specific antibody response	56–61

* (1) Sherwood *et al.*, 1990, (2) Nakajima *et al.*, 1995, (3) van Moorselaar *et al.*, 1991, (4) Kuniyasu *et al.*, 2001, (5) Kramer *et al.*, 2001, (6) McDevitt *et al.*, 2000, (7) Sinha *et al.*, 1999, (8) Deguchi *et al.*, 1986, (9) Deguchi *et al.*, 1987, (10) Agus *et al.*, 1999, (11) Saffran *et al.*, 2001, (12) Deb *et al.*, 1996, (13) Meredith *et al.*, 1994, (14) Slovin *et al.*, 1998, (15) Meredith *et al.*, 1999, (16) Schwaab *et al.*, 2001, (17) Katzenwadel *et al.*, 2000, (18) Cesano *et al.*, 1998, (19) Granziero *et al.*, 1999, (20) Ross *et al.*, 1993, (21) Gong *et al.*, 1999, (22) Guinan *et al.*, 1979, (23) Guinan *et al.*, 1982, (24) Pollard *et al.*, 1994, (25) Rini *et al.*, 2001, (26) Hrouda *et al.*, 1998a, (27) Hrouda *et al.*, 1998b, (28) Sanda *et al.*, 1994, (29) Sanda *et al.*, 1997, (30) Dreicer *et al.*, 2001, (31) Kwon *et al.*, 1997, (32) Kwon *et al.*, 1999, (33) Hurwitz *et al.*, 2000, (34) Mercader *et al.*, 2001, (35) Brasel *et al.*, 1996, (36) Maraskovsky *et al.*, 1996, (37) Ciavarra *et al.*, 2000, (38) Sanda *et al.*, 1994, (39) Hurwitz *et al.*, 2000, (40) Tjao *et al.*, 1999, (41) Simons *et al.*, 1999, (42) Yedavelli *et al.*, 1999, (43) McElrath *et al.*, 1995, (44) Kim *et al.*, 1998, (45) Kim *et al.*, 2001, (46) Mincheff *et al.*, 2000, (47) Hodge *et al.*, 1995, (48) Fong *et al.*, 1997, (49) Sanda *et al.*, 1999, (50) Eder *et al.*, 2000, (51) Tjoa *et al.*, 1999, (52) Burch *et al.*, 2000, (53) Fong *et al.*, 2001, (54) Lodge *et al.*, 2000, (55) Fong *et al.*, 1997, (56) Slovin *et al.*, 1999, (57) Jayashankar *et al.*, 1989, (58) Rovan *et al.*, 1992, (59) Firi *et al.*, 1991, (60) Fuerst *et al.*, 1997, (61) Simms *et al.*, 2000.

II. PASSIVE IMMUNOTHERAPY

A. Nonspecific

1. CYTOKINES

Cytokines are hormones produced by cells of the immune system that mediate communication between the cellular participants in an immune response. Cytokines can affect tumor growth indirectly, e.g., by inducing lytic T cells, or directly by acting on tumor cells. Administration of cytokines that can affect tumor cell growth directly is considered a passive therapy, as no host immune cells are required for an antitumor effect. The cytokines that have been explored most extensively for passive therapy are the interferons (IFN-α and IFN-γ) and tumor necrosis factor-α (TNF-α). Interferons have antiproliferative activity and can induce direct tumor cell death (Wadler, 1991). Additionally, the interferons can also increase specific immunity by causing the upregulation of surface MHC molecules on tumor cells (Wadler, 1991). TNF-α kills tumors by direct toxic effects and indirectly by effects on tumor vasculature. Coadministration of both IFN and TNF-α has been shown to have a synergistic effect on the proliferation of both androgen-dependent and androgen-independent prostate cancer cells (Nakajima *et al.*, 1995; Sherwood *et al.*, 1990). In addition, *in vitro,* Kuniyasu *et al.* (2001) demonstrated increased apoptosis in an androgen-insensitive human prostate cancer cell line, LNCaP-LN3, when the cells were treated with both the chemotherapeutic drug doxorubicin and INF-α over treatment with doxorubicin alone.

In vivo evidence that passive immunotherapy with IFN and TNF-α can have an antitumor effect in prostate cancer has largely come from animal models. For instance, subcutaneous peritumoral administration of IFN-γ and TNF-α in rats with subcutaneous tumors induced by injection of Dunning rat tumor cells was reported to have an antitumor effect (van Moorselaar *et al.*, 1991). The use of IFN and TNF-α in the clinic has been limited due to unacceptable toxicity at biologically active doses when given intravenously. However, attempts are being made to overcome this limitation by intratumor injection of the cytokines. Kramer *et al.* (2001) tested the feasibility of such treatment in prostate cancer patients. Ten patients with hormone refractory prostate cancer (HRPC) were treated with TNF-α injected locally into prostate tumor tissue at 4-week intervals combined with intermittent sc administration of INF-α2b three times per week. Although there was a low level of leakage of TNF-α into the systemic circulation, this treatment was well tolerated. Prostate tumor cell necrosis was seen in all patients, with a significant reduction of prostate volume in 9 out of the 10 patients, and in the long term, prostate-specific antigen (PSA) serum levels decreased in the majority of patients, indicating a decrease in tumor burden. Although no objective responses of metastases were seen, these results do suggest that administration of TNF-α directly into the tumor site can be effective in localized prostate cancer.

B. Specific

Specific immunotherapy makes use of antigen-specific B cells/antibodies or T lymphocytes. In passive, specific immunotherapeutic approaches, tumor antigen-specific antibodies or T cells are adoptively transferred into a recipient. These antibodies or cells then directly mediate tumor protection or regression. Before specific immunotherapy can be attempted, tumor-specific

Table II Prostate Tumor-Associated Antigens

Antigen	Possible application
Cell surface molecules	T-cell-mediated therapy, mAb therapy
PSMA[a]	
PSCA[a]	
HER-2/neu	
STEAP[a]	
Globo H	
Intracellular proteins	T-cell-mediated therapy
PAGE-1	
CAGE-7	
PAGE-4	
PSGR	
Secreted molecules	T-cell-mediated therapy, mAb therapy (less likely)
PSA[a]	
Prostase	
TMPRSS2	
PAP[a]	
MUC-1/2	
TAG-72	

[a]Expressed in current rodent models of prostate cancer.

or tumor-associated antigens must be identified that can be used as targets for such therapy.

1. PROSTATE TUMOR-ASSOCIATED ANTIGENS

The perfect prostate tumor-specific antigen would be one that is expressed solely by prostate tumor cells and not by any normal cell, such as a mutated protein. However, because such antigens are usually unique to each individual patient's cancer, these antigens are not suitable for widespread clinical applications. Short of tumor-specific antigens, the ideal prostate cancer antigens are ones that are prostate specific, not expressed in any essential organ, and expressed at high levels in prostate cancer. In addition, for antibody-based therapy, the antigen should be expressed on the cell surface so that it is susceptible to recognition by antibodies. Table II lists several antigens that have been found to be overexpressed in prostate cancer, and these antigens are discussed later. These molecules fulfill some, but not all, of the criteria for ideal prostate cancer antigens.

a. Cell Surface Molecules

Several prostate-specific or tumor-associated cell surface molecules are overexpressed in prostate cancer. These molecules are potential targets for both antibody-mediated and T-cell-mediated therapy. Most of these cell surface molecules are proteins, including prostate-specific membrane antigen (PSMA) (Horoszewicz *et al.*, 1987; Israeli *et al.*, 1995), prostate stem cell antigen (PSCA) (Klein *et al.*, 1997; Reiter *et al.*, 1998), HER-2/neu (Kuhn *et al.*, 1993; Mark *et al.*, 1999; Mellon *et al.*, 1992; Ware *et al.*, 1991), and six-transmembrane epithelial antigen of the prostate (STEAP) (Hubert *et al.*, 1999). Additionally, a cell surface carbohydrate, Globo H, has been described as a prostate cancer antigen (Slovin *et al.*, 1999).

PSMA was discovered through the generation of specific monoclonal antibodies (mAb) against membrane preparations of the prostate cancer cell line LNCaP (Horoszewicz *et al.*, 1987). One of the resulting antibodies was specific for LNCaP, as well as the epithelium of normal and malignant prostate tissue, and was later cloned and identified as PSMA (Israeli *et al.*, 1995). PSMA is a 100-kDa transmembrane glycoprotein produced by prostatic epithelium that functions as a protease and folate hydrolase. PSMA is highly overexpressed in both primary prostate tumors and metastatic lesions, and its expression is upregulated after androgen ablation therapy (Rini and Small, 2001). Levels of PSMA mRNA are also elevated in the serum of HRPC patients (Kawakami and Nakayama, 1997). In addition to prostate tissue, PSMA expression is detectable, at a much lower level than in prostate, in several normal tissues, including duodenal epithelium, renal tubular epithelium, colonic ganglion cells, and benign breast epithelium, salivary glands, and the brain (Chang *et al.*, 1999).

PSCA was found using the LAPC-4 xenograft model in an effort to identify genes associated with prostate cancer progression (Klein and Boon, 1993; Reiter *et al.*, 1998). The PSCA gene encodes a 123 amino acid protein with an amino-terminal signal sequence and a carboxyl-terminal GPI anchor sequence. PSCA is 30% homologous to the SCA-2 gene, a member of the Ly-6 family of GPI-anchored cell surface proteins (Mao *et al.*, 1996). The function of PSCA, however, is currently unknown. PSCA mRNA has been shown to be present in the prostate and at lower levels in the placenta and bladder and is highly overexpressed in many prostate cancer cell lines and clinical specimens, with a higher level of expression correlating with advanced disease (Gu *et al.*, 1999).

HER-2/neu, also known as erbB2, is an oncogenic protein that is a member of the epidermal growth factor receptor (EGFR) family (Earp *et al.*, 1995). HER-2/neu is overexpressed in 20–30% of human breast cancer and has gained much attention in breast cancer treatment, resulting in FDA approval of a mAb (Herceptin) to treat advanced breast cancer patients with HER-2/neu-positive tumors (Saffran *et al.*, 1999). HER-2/neu is also overexpressed in a large percentage of ductal carcinomas *in situ* (DCIS) and in 20–30% of ovarian cancers (Disis and Cheever, 1997; Slamon *et al.*, 1989). In normal adult tissues, HER-2/neu is known to be expressed at low levels in skin, digestive tract epithelium, breast, ovary, hepatocytes, and alveoli (Press *et al.*, 1990). Expression of HER-2/neu in prostate tissues has just been examined. Expression has been found in both normal and cancerous prostate epithelial cells, with reported positive rates of 9–33% of specimens tested, although many investigators have found expression to be far less than noted in the literature (Kuhn, 1993; Mark, 1999; Mellon *et al.*, 1992; Ware *et al.*, 1991). Some studies have suggested that HER-2/neu may play an important functional role in prostate cancer progression to androgen independence (Craft *et al.*, 1999; Yeh *et al.*, 1999).

STEAP was identified by use of a subtractive hybridization approach in the LAPC-4 xenograft model of human prostate cancer (Hubert *et al.*, 1999). The putative six-transmembrane domain, conserved between mouse and human, suggests a potential function for the protein as a channel, receptor, or transporter protein. However, the function of this protein is currently unknown. STEAP is expressed predominantly in human prostate tissue and is upregulated in multiple cancer cell lines, including prostate, bladder, colon, ovarian, and Ewing sarcoma. Little to no expression is detected in plasma membranes of normal, nonprostate human tissues, except for bladder tissue, which expresses low levels of STEAP at the cell membrane.

In addition to cell surface proteins, many carbohydrate and glycoprotein antigens are expressed on primary and metastatic prostate cancer. These include the glycolipid antigens Globo H (Slovin *et al.*, 1999) and GM2 (Slovin, 2001); the mucins MUC-1, MUC-2, and TAG-72 (Finn *et al.*, 1995; Salgaller, 2000); and mucin-related antigens Tn(c) and TF(c) (Slovin, 2001). Because only protein antigens are capable of generating a T-cell response, immunotherapeutic strategies targeting carbohydrate antigens will require antibodies.

b. Intracellular Proteins

In addition to prostate-specific cell surface molecules, several prostate-specific intracellular proteins are also overexpressed in prostate cancer. Because these proteins are not expressed on the cell surface, they would not be useful for antibody-mediated therapy. However, they are possible T-cell antigens. Many of these intracellular proteins are encoded by genes that belong to a group of genes, known as cancer testes antigens, that are normally expressed primarily in testes but that are also expressed in many cancers. The first group of genes encoding cancer testes antigens to be identified, MAGE genes, were characterized in melanoma (Marchand *et al.*, 1995). Homologues of the MAGE genes, including the CAGE and BAGE genes, have also been identified in both melanoma and other tumor types. A new family of prostate-specific cancer testes antigens homologous to the MAGE/CAGE families, named PAGE (prostate-associated gene), has also been identified (Brinkmann *et al.*, 1998; Van den Eynde *et al.*, 1995). One of these genes, PAGE-1, is expressed in prostate cancer, as well as in normal testes and placental tissue. PAGE-1 mRNA levels have been found to be fivefold higher in the LNCaP androgen-independent, metastatic cell line versus the parental androgen-dependent, nonmetastatic LNCaP cell line. A second PAGE family gene that has been identified is actually a new CAGE family member and is known as CAGE-7 (Chen *et al.*, 1998). This gene is also expressed in normal testes and placenta, as well as in prostate cancer tissue. In contrast to PAGE-1, CAGE-7 mRNA levels were found to be the same in both parental and metastatic LNCaP cell lines. A third PAGE family member, PAGE-4, is expressed in prostate, testicular, and uterine cancers, as well as in normal male and female reproductive tissues (Brinkmann *et al.*, 1998).

A new prostate-specific gene with homology to a G-protein-coupled receptor (PSGR) has been identified (Xu *et al.*, 2000). The expression of this gene, measured at the RNA level, is highly prostate tissue specific and is overexpressed in prostate cancer specimens. The predicted protein sequence of PSGR is seven transmembrane-spanning domains with homology to G-protein-coupled odorant receptors.

c. Secreted Molecules

Although cell surface or intracellular molecules are thought to make the best potential immune targets, there are secreted molecules that are also possible immunotherapeutic targets in prostate cancer. It does have to be taken into consideration, however, that because these molecules are secreted, it may be more difficult to break any immune tolerance to these proteins. In addition, these targets may not be as suitable for antibody-mediated therapy as the antibody may bind to the secreted molecule and be taken out of circulation before reaching the tumor cells.

The most well-known prostate-specific antigen is the secreted protein PSA. PSA was originally discovered as a prostate tissue-specific antigen in 1970 (Ablin *et al.*, 1970). This protein was later identified from human seminal plasma as a 34-kDa serine protease and as a member of the human kallikrein gene family (Hara *et al.*, 1971; Saffran *et al.*, 1999). PSA is produced in normal and malignant prostate epithelial cells and is normally found at high concentrations in the seminal fluid where it is thought to play a role in liquefaction of the semen (Lilja, 1985; Oesterling, 1991). In 1980, Papsidero and co-workers discovered that circulating PSA could also be found in the serum. Circulating PSA is present at very low concentrations in the serum of healthy males, but circulating PSA levels rise dramatically in patients with benign prostatic hypertrophy (BPH) and prostate cancer (Lee and Oesterling, 1995; Oesterling, 1991). Currently, serum PSA is the best available biomarker to diagnose prostate cancer and follow disease progression after treatment (Polascik *et al.*, 1999). PSA expression is retained in both androgen-dependent and metastatic androgen-independent disease, making it a possible target for immune intervention.

Two additional secreted serine proteases similar to PSA have also been identified. One, called prostase, was identified from a prostate cDNA library by subtractive hybridization and independently by a positional cloning approach (Nelson *et al.*, 1999; Yousef *et al.*, 1999). Prostase mRNA is highly expressed in normal and malignant prostate tissue and is expressed at lower levels in testis, mammary gland, adrenals, uterus, thyroid, and salivary glands (Nelson *et al.*, 1999; Yousef *et al.*, 1999). The second protease, TMPRSS2, was identified using an androgen-stimulated, LNCaP-derived mRNA to probe cDNA microarrarys (Lin *et al.*, 1999). TMPRSS2 is expressed in the basal cells of normal prostate and in epithelial cells in prostate adenocarcinoma (Afar *et al.*, 2001; Tanimoto *et al.*, 1997). Both of these proteases are potentially overexpressed in prostate cancer, making these proteins possible immunotherapy targets.

Prostatic acid phosphatase (PAP) is another prostate-specific secreted protein. PAP is an isoenzyme expressed in both rodents and humans. This enzyme was first identified in 1936 as having a phosphatase activity associated with the osteoblastic metastasis of prostate cancer (Gutman *et al.*, 1936). PAP is prostate specific, with expression detectable only in normal and cancerous prostate epithelial cells, but not in any other tissues investigated (Lam *et al.*, 1989; Sinha *et al.*, 1998; Solin *et al.*, 1990). Circulating PAP levels in the serum of cancer patients have been shown to increase progressively with the disease, and elevated levels in advanced disease have been associated with a poor prognosis (Jacobs and Haskell, 1991).

As mentioned earlier, the best animal prostate cancer model currently available is the TRAMP model. To make this model useful for testing methods of specific immunotherapy, prostate tumor-associated antigens must be identified that are expressed by both TRAMP tumors and human prostate cancers. To date, only a few such antigens have been identified. Using a subtractive hybridization technique, the authors' laboratory identified three genes that are overexpressed in TRAMP tumors that are also overexpressed in human prostate cancer, namely murine Psca (mPsca), mPsma, and mSteap (Yang *et al.*, 2001). The mRNA expression profiles of these three genes in normal tissues are similar between mouse and human. Identification of these murine genes in combination with the TRAMP model will provide an excellent preclinical model in which to evaluate antigen-specific immunotherapy strategies for prostate cancer.

Animal models are also being used to test the potential for targeting two prostate-specific secreted proteins in immunotherapy: PAP and PSA. PAP is secreted in rodents as well as in humans allowing for study in rat and murine models of prostate cancer of immunotherapy against PAP. There is no known murine PSA homologue, but human PSA transgenic mice have been engineered to allow the study of the possible use of PSA as a target of immunotherapy against prostate cancer (Wei *et al.*, 1997). This same group was able to induce a PSA-specific CTL response in these mice, demonstrating that immunization against PSA, when it is a self-antigen, is possible (Wei *et al.,* 1997). In addition, Correale *et al.* (1998) were able to induce PSA-specific CTL activity *in vivo* in HLA-A2.1/K^b transgenic mice, demonstrating that HLA-A*0201-restricted PSA T-cell responses are possible.

With prostate-specific antigens now in hand, specific immunotherapy approaches for the therapy of prostate cancers are being tested in both animal models and clinical trials.

2. MONOCLONAL ANTIBODIES

Antibodies are considered attractive antitumor agents due to their minimal toxicity. Antibodies can mediate cell death via complement fixation or antibody-dependent, cell-mediated cytotoxicity (ADCC) of cells expressing a specific antigen. Antibodies can also be used to deliver toxins to cells that only express a specific antigen. Such specific delivery of toxins to tumor cells is achieved by the conjugation of antibodies specific for a tumor-associated antigen to radioactive or other toxic moieties. In addition to the use of antibodies to directly cause the death of tumor cells, antibodies can be used to predispose tumor cells to apoptosis. For example, an antibody may bind to a growth factor receptor and block binding of a growth factor required for tumor cell survival. Such a mechanism is believed to be responsible for the effectiveness of anti-HER-2/neu mAb (see later).

Antibodies conjugated to radioactive or toxic moieties specific for multiple prostate cancer antigens have demonstrated antitumor activity in animal models. Radiolabeling of an antibody to the external domain of PSMA has been shown to have antitumor effects in both cell lines and murine models (McDevitt *et al.,* 2000). A polyclonal rabbit anti-PSA IgG antibody, conjugated with a labeled derivative of 5-FU, was tested *in vivo* in nude mice for its ability to target PSA-positive LNCaP or PSA-negative DU145 tumors (Sinha *et al.,* 1999). The antibodies localized and were toxic to LNCaP but not to DU145 tumors, demonstrating that even though PSA is a secreted protein, antibodies against it can localize to prostate epithelial cells and effectively deliver therapeutic drugs. In contrast, PAP-specific monoclonal antibodies conjugated with either methotrexate or adriamycin were able to inhibit growth of LNCaP cells *in vitro,* but were unable to inhibit the growth of established LNCaP tumors (Deguchi *et al.,* 1986, 1987). Although antibodies were seen at the tumor site, their numbers may not have been sufficient to cause tumor destruction. This result may have been due to an inability of the antibodies to infiltrate the tumor because of the high interstitial pressure that is present in solid tumors. Alternatively, the antibodies may have been ineffective due to the binding of circulating PAP, resulting in clearance of the antigen–antibody conjugate.

Herceptin, the mAb specific for HER-2/neu used in the treatment of breast cancer, may be useful in the treatment of prostate cancer as well. In this case, suppression of tumor growth would most likely be mediated through binding of the antibody to HER-2/neu molecules on the cell surface blocking binding of a growth factor required for tumor cell survival. Herceptin was able to inhibit the growth of androgen-dependent but not androgen-independent xenografts in mice, indicating that there may be a necessity for signaling through the androgen receptor for an effective Herceptin response (Agus *et al.*, 1999). In another study, Morris *et al.* (2002) found that trastuzumab and paclitaxel were ineffective in prostate cancer patients whose tumors expressed Her-2/neu.

Saffran *et al.* (2001) demonstrated that anti-PSCA mAb were able to inhibit formation of both androgen-dependent LAPC-9 and androgen-independent PC3-PSCA tumor xenografts in severe combined immunodeficient (SCID) mice. Anti-PSCA mAb were also able to significantly slow down the growth of established tumors and prolong survival of tumor-bearing mice. The treatment also resulted in a near complete inhibition of lung metastasis formation in tumor-bearing mice. The mechanism by which the anti-PSCA mAb exerted their effect on prostate cancer cells is unknown.

Antibodies conjugated to radioactive or toxic moieties specific for multiple prostate cancer antigens have also been tested in clinical trials. Results of a phase I trial of therapy of HRPC patients with a yttrium (^{90}Y)-labeled PSMA-specific murine antibody have been reported (Deb *et al.*, 1996). Although the treatment was well tolerated, no clinical response was observed in these patients. Another series of studies involved the use of a radioactively (^{131}I) labeled antibody specific for TAG-72 along with INF-γ or IFN-α (Meredith *et al.*, 1994, 1999; Slovin *et al.*, 1999). Again, no objective clinical responses have been observed with this therapy.

Katzenwadel *et al.* (2000) have described the generation of a bispecific antibody with specificity for both PSA and the T-cell-signaling-associated molecule CD3. Such an antibody would theoretically be able to increase the delivery of T cells to the tumor site. The function of these antibodies has been demonstrated in a cytotoxicity assay with PSA-expressing tumor cells as targets and preactivated human peripheral blood mononuclear cells (PBMC) as effector cells. In addition, this antibody was shown *in vivo* in nude mice to significantly reduce tumor growth when both the antibody and effector T cells were adoptively transferred into the mice.

3. T-CELL ADOPTIVE THERAPY

T-cell adoptive therapy involves the infusion of tumor-specific T cells that have been generated *in vitro* into tumor-bearing recipients. Proof of principle of adoptive T-cell therapy was first achieved with the adoptive transfer of specific T cells into postallogeneic bone marrow transplant patients with cytomegalovirus (CMV) specific cytotoxic T lymphocytes (CTL) (Riddell *et al.*, 1992). Persistent anti-CMV immunity was detected in the blood of the recipients, demonstrating that adoptive T-cell therapy was safe and functional.

Preclinical animal models of various tumor types have demonstrated the regression of established tumors using adoptive T-cell therapy. In a prostate cancer model, Cesano *et al.* (1998) tested the effectiveness of a human T-cell line (TALL-104), which has MHC nonrestricted cytotoxic activity specific for a broad range of tumors across several species, to inhibit the growth of the human prostate cancer cell line DU-145 in SCID mice. Multiple transfers of these T cells into mice bearing small subcutaneous established tumors resulted in a reduction of local tumor growth and complete prevention of pulmonary metastases. Another group, Granziero *et al.* (1999), successfully used adoptive T-cell therapy against spontaneous tumors in the TRAMP model. The T cells used were obtained by activating splenocytes from syngeneic C57BL/6 mice *in vitro* with Tag-expressing tumor cells. These cells were then adoptively transferred into TRAMP mice starting at 10 weeks of age—a time when tumor development would be expected to have begun in these mice. The authors reported a significant reduction in tumor progression in these treated mice compared to untreated TRAMP mice. In addition, these mice had sustained immunity to Tag.

Although T-cell adoptive therapy has not been explored to a great extent in human studies, a few studies have demonstrated the feasibility of using adoptively transferred T cells as therapy in prostate cancer. In a pilot clinical study, Ross *et al.* (1993) treated HRPC patients with autologous T cells that had been activated *in vitro*. The therapy was determined to be feasible and safe, and transient serum PSA level reductions were seen, demonstrating a decrease in tumor burden. In another study, Gong *et al.* (1999) tested *in vitro* the effectiveness of using T cells genetically modified to contain an artificial receptor specific for a tumor antigen against prostate cancer. These researchers constructed a zeta chain fusion receptor specific for PSMA and transduced T cells obtained from prostate cancer patients with this fusion protein. These T cells were able to lyse prostate cancer cell lines, suggesting a potential use of these T cells in adoptive transfer therapy of prostate cancer patients.

III. ACTIVE IMMUNOTHERAPY

A. Nonspecific

Therapies involving nonspecific, active immunotherapy aim to induce a tumor antigen-independent inflammatory response with the aid of effector cells from the host's own immune system.

1. MICROBIAL PRODUCTS

The earliest immunotherapy tested to treat advanced prostate cancer was a nonspecific, active type of immune therapy. Namely, adjuvant therapy with *bacillus Calmette–Guerin* (BCG) (Guinan *et al.*, 1979, 1982). BCG activates macrophages and dendritic cells (DC) and is considered a possible antitumor adjuvant. BCG treatment in rats with established adenocarcinomas of the prostate has been shown to decrease the number of lung metastases compared to control untreated rats (Pollard and Luckert, 1994). However, the success of conventional BCG therapy in cancer patients has been limited. Its use is being reconsidered as an adjuvant with antigen-specific vaccines (see later).

The use of heat-killed *Mycobacterium vaccae* is also being explored in the treatment of prostate cancer. *M. vaccae* has been shown to be a potent stimulator of both $CD8^+$ and $CD4^+$ T cells in preclinical tuberculosis studies (Rini and Small, 2001). In rats, treatment with *M. vaccae* alone was unable to prevent the outgrowth of transplantable prostate tumors or reduce the size of established tumors (Hrouda *et al.*, 1998b). However, use of this bacterial preparation as an adjuvant along with an autologous tumor cell vaccine was effective in delaying the growth of established tumors in this model. These results suggest that *M. vaccae* is likely to be useful as an adjuvant, but not as a stand-alone agent. However, a clinical trial in HRPC patients with heat-killed *M. vaccae* as a single agent has been performed (Hrouda *et al.*, 1998a). Two of 10 patients treated in this study showed a decline in the PSA serum level, suggesting a decrease in tumor burden. There was also an increase in these patients in the percentage of IL-2- and IFN-γ-producing T cells along with a decline in IL-4-producing T cells, suggesting an immunological response. Future clinical trials that use *M. vaccae* in an adjuvant setting are likely.

2. CYTOKINES

One of the first cytokines tested for use in immunotherapy of cancer was interleukin-2 (IL-2). Because IL-2 has no direct impact on cancer cells, the effect of IL-2 on cancers *in vivo* is believed to be due to its ability to expand T-cell populations with antitumor activity. IL-2 treatment is being explored as a therapy for a variety of cancer types and is currently the most effective treatment for metastatic renal cell carcinoma (Heinzer *et al.*, 2001). The use of IL-2 in treatment against prostate cancer is currently being explored in both animal models and clinical trials. Systemic administration of IL-2 has been shown to decrease tumor growth and increase animal survival in multiple prostate cancer models (Henriksson *et al.*, 1992; Kocheril *et al.*, 1999; Triest *et al.*, 1998). However, because substantial toxicity can occur with systemic IL-2 treatment (Huland *et al.*, 1997), animal studies are also being done to determine whether the local intratumor administration of IL-2 can also be effective against prostate cancer growth. An IL-2 depot implanted directly next to the tumor site was able to significantly reduce the growth of established Dunning prostate tumors in rats (Hautmann *et al.*, 1999), and delivery of IL-2 with microosmotic pumps reduced the growth of the HRPC cell line Mat LyLu in rats (Hautmann *et al.*, 2000). No toxicity was observed during these treatments. These data suggest that local intratumoral IL-2 therapy can be an effective therapy against prostate cancer with less toxicity than systemic therapy.

Results of a pilot study involving the subcutaneous administration (Maffezzini *et al.*, 1996) of IL-2 and IFN-α to 15 HRPC patients have been reported. Some partial responses and PSA serum level reductions were seen, suggesting an antitumor effect of these cytokines. In a phase I clinical trial, a DNA–lipid complex encoding the IL-2 gene was administered intraprostatically in 24 patients with locally advanced prostate cancer. The therapy was well tolerated, and evidence of immune activation following therapy was observed. Namely, an increase in T-cell infiltration was seen on the immunohistochemical analysis of tissue samples from the injected tumor sites. Transient decreases in serum PSA levels were observed in 16 of 24 patients, suggesting a decrease in tumor burden with IL-2 treatment.

Another cytokine with potential application in immunotherapy for cancers is IL-12. IL-12 has been shown to be crucial in driving the differentiation of T cells to a T helper 1 (Th1) phenotype. Both $CD4^+$ and $CD8^+$ T cells of this phenotype produce high amounts of IFN-γ, and $CD8^+$ cytotoxic T lymphocytes with a Th1-like phenotype are also highly lytic. Because high amounts of IFN-γ and CTL with great lytic capability are believed to have great antitumor activity, IL-12 is a logical cytokine to test in immunotherapy for cancer. In addition to its role in modulating tumor-specific T-cell differentiation, IL-12 may also contribute to an antitumor effect by interfering with angiogenesis via production of IFN-inducible protein-10 (IP-10) (Angiolillo *et al.*, 1996; Coughlin *et al.*, 1998).

Treatment of established DU-145 prostate tumors in the lungs of SCID mice with a combination of transplanted human effector cells and a fusion protein, consisting of IL-12 and an antibody specific for human epithelial cell adhesion molecule (therefore specific for the human DU-145 cells), was able to drastically reduce the number of lung metastases. In the RM-1 mouse model of prostate cancer, adenovirus-mediated IL-12 gene therapy significantly inhibited both primary tumors and metastatic lesions. These studies demonstrate that treatment with IL-12 can be effective against prostate cancer in murine models, warranting clinical trials with this cytokine. IL-12 has yet to be tested in the clinic for this disease, however.

In addition to the use of cytokines as a way to boost a tumor-specific T-cell response, the possible use of cytokines to boost the innate immune system is also being explored in tumor immunotherapy. One cytokine potentially useful in heightening the innate immune response against tumors is IL-15. IL-15 plays a critical role in the development, survival, and function of natural killer (NK) cells (Carson *et al.,* 1997; Mrozek *et al.,* 1996; Ogasawara *et al.,* 1998; Ohteki *et al.,* 1998) and is important in innate immune INF-γ production (Fehniger *et al.,* 2000). NK cells are innate immune cells that lyse cells lacking class I MHC expression. Because many tumor cells downregulate the expression of MHC molecules (discussed later in this review), the induction of NK cell-mediated antitumor immune responses is believed to be of potential benefit in immunotherapy against cancers.

Suzuki *et al.* (2001) explored the potential antitumor effect of IL-15 in a prostate cancer model. The human prostate cancer cell line PC-3 was transfected with a secretable form of the IL-15 gene, and the growth of these tumor cells in nude mice was tested. Although the PC-3/IL-15 and PC-3/mock transfectants grew similarly *in vitro,* growth of the IL-15-secreting tumor cells was delayed significantly compared to the mock-transfected tumor cells *in vivo,* with many of the mice completely rejecting the IL-15-secreting tumor cells. NK cell depletion via the anti-asialo GM1 antibody completely restored growth of the IL-15-secreting tumor cells *in vivo,* demonstrating a requirement for NK cells for the antitumor effect. These results suggest that IL-15 could be useful in immunotherapy of prostate cancer. IL-15 has not yet been brought to the clinic, however.

In recent years, interest has accumulated in the possible use of another cytokine known as granulocyte–monocyte colony-stimulating factor (GM-CSF) in cancer immunotherapy. This interest is due to the ability of GM-CSF to enhance the expansion of macrophages, neutrophils, and eosinophils (Rivas *et al.,* 1998) and to promote the migration, development, and longevity of DC (Dranoff *et al.,* 1993). In addition, GM-CSF can directly mediate antitumor activity by activating macrophages to release TNF-α (Salgaller, 2000).

The immunotherapeutic potential of GM-CSF in prostate cancer has been demonstrated in a rat model. Studies by Sanda *et al.* (1994) showed that GM-CSF-transfected rat prostatic adenocarcinomas grew more slowly than parental tumors. Treatment of rats with GM-CSF prior to tumor challenge has also been shown to delay tumor onset and to increase survival time (Sanda, 1997). In addition, GM-CSF has also been used successfully as a vaccine adjuvant in animal models (discussed later in this review) and is probably better in this capacity than as a stand-alone agent.

GM-CSF as a stand-alone agent has been tested in clinical trials in HRPC patients (Dreicer *et al.,* 2001). Toxicity was minimal, and a majority of the patients experienced a mild decrease in PSA serum levels when on GM-CSF therapy that subsequently increased when therapy was discontinued. However, no objective clinical responses were observed. Because these results demonstrate a modest biologic activity of GM-CSF alone in prostate cancer, the majority of clinic trials involving GM-CSF are now investigating its possible use as a vaccine adjuvant.

3. SUICIDE GENE THERAPY

Suicide gene therapy involves the genetic modification of a cell to make it vulnerable to the administration of an otherwise nontoxic prodrug (Hassan *et al.*, 2000). The most common example is the herpes simplex virus thymidine kinase (HSVtk) enzyme in combination with ganciclovir. This therapy can result in the shrinkage of tumor mass even when only a small percentage of the tumor cells express HSVtk through a "bystander effect" (Gagandeep *et al.*, 1996). Although such therapy is not immunotherapy per se, a competent immune system appears necessary for the full "bystander effect" of suicide gene therapy (Gagandeep *et al.*, 1996; Hassan *et al.*, 2000).

In a mouse model of prostate cancer, NK cells have been identified as the mediator of tumor destruction following an adenovirus-mediated expression of HSVtk and ganciclovir administration (Hall *et al.*, 1998). Hassan *et al.* (2000) reported superior results in a mouse prostate cancer model with combination therapy involving a HSVtk-expressing adenovirus, an IL-12-expressing adenovirus, and ganciclovir. This treatment resulted in a reduction of large tumor burdens, and enhanced NK lytic activity correlated with the tumor destruction.

Herman *et al.* (1999) reported results of a phase I clinical trial in prostate cancer patients with suicide gene therapy. The treatment consisted of injection directly into the prostate of a replication-defective adenovirus containing the HSVtk gene followed by iv administration of ganciclovir. Three of 18 patients in this trial demonstrated an objective response, suggesting a potential usefulness of this type of therapy in prostate cancer treatment.

B. Specific

The ultimate goal of active, specific immunotherapy is the vaccination of patients to induce long-lived, tumor-specific immunity capable of rejecting active disease as well as protective immunological memory. Implicit to achieving this goal is induction of a tumor-specific T-cell response capable of mediating tumor cell death. In addition, because functional DC capable of efficiently presenting tumor antigen to T cells are probably required for the induction of tumor-specific T cells, the presence of functional DC in cancer patients is critical to active, specific therapy.

At least two hurdles must be overcome before induction of an effective T-cell response in prostate cancer patients is likely. First, because all of the prostate cancer antigens that are known are self-proteins, T-cell tolerance to these proteins is to be expected. Therefore, mechanisms for breaking self-tolerance need to be elucidated before effective T-cell-inducing vaccination is likely to be achieved in prostate cancer patients. Second, because moderate immunosuppression has been noted in prostate cancer patients, especially those with advanced stages of the disease (Healy *et al.*, 1998; Herr, 1980; Salgaller *et al.*, 1998) (discussed later in this review), ways to strengthen these patients' immune systems need to be determined. Several strategies are being tested in both animal models and clinical trials to overcome these two hurdles and to induce an effective prostate-specific T-cell response in prostate cancer patients, and many of these strategies are discussed here.

1. ENHANCEMENT OF ENDOGENOUS T-CELL ACTIVITY

a. CTLA-4 Blockade

The essential mechanisms involved in the activation and inhibition of T cells have been elucidated recently. It is now accepted that complete T-cell activation requires at least two signals. The primary signal for activation is ligation of the T-cell receptor (TCR) with a major histocompatibility complex (MHC) molecule containing an antigenic peptide. In addition to this signal, costimulatory signals are usually required for complete T-cell activation and acquisition of effector function. The best-described costimulatory signal is ligation of CD28 on T cells by B7-1 or B7-2 (CD80/CD86) expressed on professional antigen-presenting cells (APC). Whereas costimulation by ligation of CD28 by B7-1/2 is a T-cell activation signal, ligation of the CD28 homologue, CTLA-4, by B7-1/2 is a T-cell inhibitory signal. Therefore, the result of TCR signaling is dependent on the competing stimulatory and inhibitory interactions of B7-1/2 with CD28 and CTLA-4, respectively. Antibodies that block CTLA-4 and B7 interactions have been demonstrated to augment T-cell responses (Awwad and North, 1989). The possibility that such antibodies may be able to be used to enhance prostate tumor-specific T-cell responses is being explored in animals, but has not yet been tested in prostate cancer patients.

Kwon *et al.* (1997) demonstrated that *in vivo* antibody-mediated blockade of CTLA-4/B7 interactions promoted regression of TRAMP-C1-transplanted tumors. CTLA-4 blockade has also been shown to be effective at reducing metastatic relapse following primary tumor resection in the TRAMP-C2 tumor resection/metastasis model (Kwon *et al.*, 1999). In addition, Hurwitz *et al.* (2000) demonstrated that an effective immune response against primary prostate tumors in TRAMP mice could be generated using CTLA-4 blockade along with an irradiated tumor cell vaccine.

b. Androgen Ablation

Androgen ablation is a routinely used palliative treatment of metastatic prostate cancer that induces rapid involution of hormone-dependent cancerous prostate tissues. Data show that this treatment may not only cause the destruction of tumor cells, but may also induce a T-cell response against the tumor cells. Mercader *et al.* (2001) found that androgen ablation therapy in human subjects induced T-cell infiltration into benign prostate glands as well as prostate tumor tissue. T cells found in the prostate tissues were predominately $CD4^+$ cells, with a smaller number of $CD8^+$ T cells also present. T cells in the prostate had restricted TCR $V\beta$ gene usage, suggesting a clonal response. These data suggest androgen ablation may cause prostate-specific T-cell-mediated inflammation that may lead to a break in T-cell tolerance to prostate-specific antigens. That androgen ablation induces infiltration into the prostate suggests a potential for this type of therapy to prime prostate-specific T-cell responses that might be enhanced further by other immunotherapies, such as CTLA-4 blockade or specific, active immunization.

2. IMPROVING APC FUNCTION

It is believed that in order for an effective tumor-specific immune response to be initiated, professional APC need to take up, process, and present tumor antigens to T cells (Huang *et al.*, 1994, 1996). However, moderate immunosuppression has been noted in prostate cancer patients, especially those with advanced stages of the disease, including apoptotic death of the best APC, DC (Healy *et al.*, 1998; Herr, 1980; Salgaller *et al.*, 1998). Therefore, strategies for improving APC function in prostate cancer patients are now being tested.

As described earlier, GM-CSF is being tested for use in cancer immunotherapy partly due to its ability to promote the migration, development, and longevity of DC. In addition, Flt3-Ligand (Flt3-L), a recently described member of a small family of growth factors that stimulate the proliferation of hematopoietic stem cells, is being tested for its ability to increase DC numbers in tumor-bearing hosts. *In vivo* administration of Flt3-L in mice has been shown to dramatically increase the number of functional DC that accumulated in the spleen and lymphoid tissue (Brasel *et al.*, 1996; Maraskovsky *et al.*, 1996). Ciavarra *et al.* (2000) demonstrated in the transplantable TRAMP-C1 murine model of prostate cancer that administration of Flt3-L could delay growth of an established tumor in male mice. Flt3-L has not yet been tested in prostate cancer patients.

3. INDUCTION OF A T-CELL RESPONSE

It is not known what the best vaccination method is to induce an effective tumor-specific T-cell response. Therefore, multiple vaccination strategies are being tested, and many of these strategies are described here. As mentioned earlier, one therapeutic challenge for the induction of an effective tumor-specific T-cell response using these antigens is the possible need to overcome immune tolerance to these normal prostate antigens. One potential method of breaking T-cell tolerance that seems to have promise is immunization with xenogeneic proteins of human homologues. Xenoantigens may be effective because they are "altered self" proteins with enough difference from self-antigens to be immunogenic, but with enough similarity to allow reactive T cells to be reactive against self-antigens as well. Examples of effective immunization against prostate cancer with xenoantigens are discussed later.

a. Genetically Engineered Tumor Cells as Vaccines

Genetic-based immunizations are being explored as a way to enhance the immunogenicity of tumor cells and to induce a tumor-specific T-cell response. In this therapy, patients are vaccinated with tumor cells that have been modified genetically to express cytokines.

Multiple immunization strategies involving genetically modified tumor cells as vaccines are being tested in animal models. Some of these strategies are listed here. GM-CSF-secreting cancer cell vaccines, generated by the introduction of the GM-CSF gene into cancer cells *in vitro,* have been shown to induce tumor-specific immune responses in animal models of prostate cancer (Sanda *et al.,* 1994). Tjoa *et al.* (1999) showed that IL-2-transfected rat prostate cancer cells also induced antitumor activity. In the Dunning rat prostate carcinoma model, vaccination with irradiated prostate carcinoma cells transduced with DNA encoding GM-CSF, IL-2, or IFN-γ was able to prolong the survival of tumor-bearing animals. Murine RM-1 prostate tumor cells infected by a nonreplicating canarypox vector, ALVAC, with cytokine recombinants have also shown effectiveness in mice. As single agents, ALVAC–IL-2, ALVAC–IL-12, ALVAC–GM-CSF, and ALVAC–TNF-α were effective in partially inhibiting tumor outgrowth. As a combination therapy of ALVAC–TNF-α with ALVAC–IL-2, ALVAC–IL-12, or ALVAC–GM-CSF, tumor outgrowth inhibition was optimized. Of particular interest, Hurwitz *et al.* (2000) were able to reduce the incidence and severity of prostate tumors in TRAMP mice with a vaccine consisting of the anti-CTLA-4 antibody and irradiated TRAMP-C1 and TRAMP-C2 cells transduced to express GM-CSF.

The use of genetically modified tumor cells as vaccines in prostate cancer patients is also beginning to be explored. A study was performed in which men who were found to have metastatic disease while undergoing prostatectomy were treated with irradiated GM-CSF-secreting autologous prostate carcinoma cells transduced *ex vivo* with a GM-CSF vector (Simons *et al.,* 1999). Both T-cell and B-cell responses were elicited against polypeptides generated from the LNCaP prostate cell line in these patients, suggesting these patients had generated immune responses against prostate cancer-specific or associated antigens. The researchers found that a major difficulty with this type of treatment was the low yield of autologous, transduced prostate cancer cells recovered from cell culture and concluded that this approach was clinically impractical for large phase II studies to assess efficacy. Allogeneic prostate cancer vaccines, prepared from prostate cancer cell lines modified genetically to secrete high levels of GM-CSF, may offer a solution to this clinical development problem. Preclinical studies have suggested that because antigens from irradiated cancer cells are presented to T cells by host APC, tumor cells used for vaccination do not necessarily need to be MHC matched with the host to elicit a tumor-specific T-cell response. However, there is a possibility that vaccination with allogeneic tumor cells may lead to a dominant allo-specific response rather than a prostate-specific response, deviating the immune response away from prostate-specific or prostate tumor-specific antigens.

b. Whole Protein/Peptide Vaccination

With the identification of prostate-specific antigens it is now possible to design antigen-specific vaccines that do not rely on tumor cells. While one immunization strategy is to vaccinate with an entire antigenic protein, another strategy is to vaccinate with defined HLA-binding peptides encoded by an antigenic protein. The rationale for immunization with such peptides is that $CD8^+$ and $CD4^+$ T cells do not recognize whole protein, but rather antigenic peptides in MHC class I and class II molecules, respectively. Advantages for immunizing with peptide rather than whole protein include that peptides are relatively easy to manufacture and store, no infectious agents are involved in their generation, and any potential oncogenic or deleterious biological activity of the whole protein can be avoided. In attempts to begin to explore the possible use of peptide-based vaccinations, antigenic peptides encoded by prostate-associated antigens, including PSMA (Murphy *et al.*, 1996; Tjoa *et al.*, 1996) and PSA (Alexander *et al.*, 1998; Correale *et al.*, 1997, 1998), that bind to HLA molecules are being defined.

One vaccination approach is to immunize with an antigenic protein or peptide emulsified in an oil-based adjuvant. Animal studies involving vaccination with incomplete or complete Freund's adjuvant have demonstrated the ability of such vaccination to generate tumor-specific CTL (Feltcamp *et al.*, 1993; Mandelboim *et al.*, 1995; Zhang *et al.*, 1996). However, the efficacy of this approach in a prostate cancer model has not been tested. As an alternative to using defined antigens, heat shock proteins (HSP) isolated from tumor cells, which have tumor antigenic peptides bound to them, have been used successfully to generate tumor-specific T-cell responses (Srivastava *et al.*, 1994; Suto and Srivastava, 1995). In the Dunning rat prostate cancer model, vaccination with tumor-derived HSP delayed both the incidence and the growth of tumors (Yedavelli *et al.*, 1999).

Multiple clinical trials have been described with a PSA-specific vaccine known as Onco Vax-P (Salgaller, 2000). This vaccine consists of the PSA protein in carrier liposomes with lipid A as an adjuvant. Clinical trials differed in the method of administration and the adjuvant(s) administered along with the basic vaccine. DTH responses and high antibody titers were induced in at least 40% of immunized patients when Onco Vax-P was delivered via intradermal injection together with one of the following: BCG, im in a mineral oil emulsion, sc with GM-CSF, or sc with GM-CSF and IL-2. These studies demonstrated that this vaccine is feasible and safe and can induce humoral and cellular immunity. This vaccine is now being tested in patients with less advanced disease to determine if the vaccine can favorably influence the clinical outcome.

One strategy being tested to enhance the immunogenicity of a peptide encoded by a self-antigen such as PSA is immunization with agonist peptides that are potentially more immunogenetic than the naturally occurring peptide. Terasawa *et al.* (2002) described the design of an agonist PSA peptide. The peptide was generated by the mutation of MHC anchor residues of the naturally occurring peptide. This peptide had enhanced binding to the HLA-A2 molecule and enhanced stability of the peptide/MHC complex compared to the corresponding native peptide. T cells stimulated with DC pulsed with the agonist peptide produced higher levels of IFN-γ compared to DC pulsed with the native peptide, and T-cell lines generated with the agonist peptide were able to lyse human prostate cancer cells expressing native PSA in an MHC-restricted manner. In addition, vaccination of HLA-A2/K^b transgenic mice with DC pulsed with the agonist peptide generated higher levels of T-cell activation compared with vaccination with DC pulsed with the native peptide.

c. Naked DNA Vaccination

DNA immunization delivers DNA constructs encoding a specific antigen into the host, with the host then producing the antigenic protein. Immunization of both mice (Kim *et al.*, 1998) and rhesus macaques (Kim *et al.*, 2001) with a DNA vaccine construct that encoded the human PSA gene resulted in an induction of PSA-specific humoral and T-cell responses. However, the tumor-rejection ability of these immune responses was not tested.

Vaccination of prostate cancer patients in variable disease states with DNA has also been performed. A vector containing the cDNA encoding the extracellular portion of PSMA, CD86, or both was administered to patients by intradermal injection (Mincheff *et al.*, 2000). Some patients additionally received GM-CSF with immunization. The highest percentage of delayed-type hypersensitivity (DTH) responses was observed in patients that had been immunized with both PSMA and CD86 DNA along with GM-CSF, and PSA serum level declines were observed in some patients, indicating a decrease in tumor burden following vaccination.

d. Recombinant Viral Vaccines

Multiple forms of recombinant viral vaccines are being tested for their effectiveness against prostate cancer. For instance, in a nonhuman primate model, Hodge *et al.* (1995) were able to measure PSA-specific IgM antibodies and long-lasting PSA-specific T-cell responses following vaccination with a recombinant vaccinia virus expressing human PSA. In a rodent model, Fong *et al.* (1997) demonstrated in male rats that T-cell tolerance to PAP could be overcome by immunization with xenogeneic PAP. Immunization with recombinant vaccinia expressing human PAP, but not rat PAP, generated a CTL response and tissue-specific prostatitis. The CTL generated were also able to lyse a rat prostate tumor cell line *in vitro*.

Immunization with an adenoviral vector containing cDNA encoding the extracellular portion of PSMA has also been described (Mincheff *et al.*, 2000). In a phase I clinical response, all patients immunized with the PSMA adenoviral vector developed PSMA-specific DTH responses, and PSA serum level declines were observed in some patients, indicating an effect of the vaccine on tumor growth.

Two phase I clinical studies of immunization of prostate cancer patients with a recombinant vaccinia virus containing the DNA encoding the PSA gene (rv-PSA) have been reported (Eder *et al.*, 2000; Sanda *et al.*, 1999). The first study, performed by Sanda *et al.* (1999), demonstrated the safety and feasibility of this approach, but the ability to stimulate an anti-PSA response was not demonstrated. In a second study by Eder *et al.* (2000), stable disease was achieved in 14 of 33 patients for at least 6 months. A cohort existed in this study that received GM-CSF along with the rv-PSA. Increases in PSA peptide-specific T-cell numbers were observed in 5 of the 7 HLA-A2$^+$ patients in this cohort, and 4 of these 5 patients had stable PSA levels for 6–11+ months. These results suggest that vaccination with the rv-PSA was able to induce PSA-specific immune responses and to inhibit tumor growth in some patients.

e. Dendritic Cell Vaccines

Immunization with autologous DC is a potentially powerful vaccination approach. This type of immunization may not rely on effective antigen presentation by host APC, which may be important since, as mentioned earlier, APC function in tumor-bearing hosts is often compromised. Expansion of DC from human peripheral blood has been made possible, allowing for the potential use of DC in vaccination strategies in humans. DC can be pulsed with peptide, whole protein, or transfected/transduced with DNA or RNA encoding tumor antigens and used as vaccines.

Multiple mouse studies have demonstrated effective immunization with antigen-pulsed DC, leading to tumor-specific immunity and/or protection against tumor challenge *in vivo,* although to date no such vaccination has been reported in the literature in a prostate tumor animal model.

Two studies have been described in which HRPC patients have been treated with autologous DC that had been exposed *in vitro* to recombinant PAP and GM-CSF (Burch *et al.,* 2000; Small *et al.,* 2000). In the first study, a significant increase in PAP-specific T cells and an antibody response against PAP was observed in the peripheral blood of patients treated with such DC. T cells collected after treatment (but not before) were found to secrete IFN-γ in response to PAP. Although a few patients experienced significant declines in PSA serum level, no objective regression of disease was observed. The second study involved vaccination with autologous DC pulsed with mouse PAP (Fong *et al.,* 2001). These researchers utilized a xenogeneic PAP, rather than human PAP, due to their preclinical data showing that only vaccination with a xenogeneic PAP peptide was able to break immune tolerance in mice (Fong *et al.,* 1997). Twenty-one patients were enrolled in the study and all tolerated the treatment with minimal side effects. All of the patients developed a T-cell response to mouse PAP. Half of the patients also developed T-cell responses to human PAP, demonstrating a break in tolerance to this self-antigen. Six of the patients that developed a human PAP-specific T-cell response had clinical stabilization of their previously progressing prostate cancer. These results demonstrate that immunization with a xenoantigen can break tolerance to a self-antigen in patients.

The results of phase I and phase II trials of treatment of HRPC patients with HLA-A2-binding PSMA peptide-pulsed DC have been reported by Tjoa *et al.* (1999). A tumor-specific T-cell response was observed in the majority of patients treated and some clinical responses were observed. However, although the peptides used were clearly HLA-A2 binders, HLA-A2-negative patients were among the clinical responders. These results call into question whether the clinical responses were mediated through T cells specific for the peptides that were used for immunization. In addition, there was no change in peptide-specific T-cell cytokine production or cytotoxicity as a result of treatment. In another phase II clinical trial with PSMA-pulsed autologous DC, it was reported that as many as 30% of the patients achieved clinical benefit, based on PSA serum level changes and other established criteria (Lodge *et al.,* 2000). However, although occasional immune reactivity to PSMA peptides was detected, it did not correlate with a better patient outcome. What did correlate with patient outcome was overall immunocompetence prior to treatment.

Heiser *et al.* (2001) demonstrated *in vitro* the ability to use RNA-transfected DC to stimulate prostate-specific T-cell responses. In this study, mRNA taken from human prostate cancer specimens by use of laser capture microdissection was amplified and loaded into DC. These DC were able to stimulate a potent polyclonal T-cell response from autologous T cells *in vitro*. The potency of the response was suggested by experiments demonstrating that this polyclonal T-cell response was more effective then PSA-specific CTL to recognize and lyse tumor targets. The polyclonal CTL recognized both tumor-specific antigens and antigens expressed by nonmalignant prostatic tissue. These autoreactive T cells were found to be exclusively specific for PSA, and not for other shared antigens, suggesting an immunodominant role of PSA in the prostate-specific antitumor immune response. This study provides a preclinical rationale for further investigation of this type of treatment for prostate cancer patients.

4. ANTIBODY-INDUCING VACCINES

Murine studies have demonstrated the ability to induce high-titer antibodies against Globo H via immunization with Globo H conjugated to keyhole limpet hemocyanin (KLH) and administered with the immunologic adjuvant QS-21 (Slovin *et al.*, 1999). All mice made high-titer IgM and IgG responses against Globo H, and sera from these mice reacted with Globo H-expressing cells *ex vivo*, demonstrating the generation of functional Globo H-specific antibodies following vaccination. Based on these preclinical results, a phase I clinical trial has been performed in which prostate cancer patients were vaccinated with Globo H conjugated to KLH (Slovin *et al.*, 1999). Anti-Globo H antibody responses, predominantly IgM, were demonstrated in the majority of vaccinated patients, and sera from half of the patients showed an increase in complement-mediated lysis against Globo H^+ cell lines. However, the only clinical response seen was a decreasing slope of PSA serum level rise in two of five patients with biochemical-only disease who did not receive subsequent hormone therapy.

An antibody-inducing vaccine against lutenizing hormone-releasing hormone (LHRH) caused drastic atrophy of the prostate in rodents (Jayashankar *et al.*, 1989; Rovan *et al.*, 1992) and monkeys (Giri *et al.*, 1991). The effectiveness of this vaccination is likely due to the downregulation of gonadotropins and testosterone and consequently the atrophy of testosterone-dependent organs such as the testes and prostate. Studies in rats implanted with androgen-sensitive and androgen-insensitive Dunning sublines have shown that this vaccine fully suppresses the growth of the androgen-sensitive cells, but only partially suppresses the growth of the androgen-insensitive cells (Fuerst *et al.*, 1997). Similarly, in an effort to find an alternative means of achieving androgen deprivation, an antibody-inducing vaccine against gonadotrophin-releasing hormone (GnRH) has also undergone a phase I clinical trial (Simms *et al.*, 2000). Twelve patients with prostate cancer in whom hormonal therapy was indicated were recruited. Prior to immunization, none of the patients had detectable GnRH-specific antibodies, whereas

11 of the 12 patients developed GnRH-specific antibodies following vaccination. In 4 of the patients, immunization resulted in castration levels of serum testosterone and in a decrease in serum PSA levels. There was a quantitative correlation between antibody titers and a decrease in PSA serum levels. The effect of the antibodies is thought to mainly be through the induction of androgen deprivation. However, because high-affinity GnRH receptors are found in a high proportion of prostate cancers, it is possible that the antibodies have a direct effect on prostate tumor cells. This type of therapy will most likely not be used as a means of castration, as the current surgical and medical methods of castration are more effective. However, the fact that GnRH receptors are expressed on the surface of prostate cancers suggests a possible future immunotherapeutic use of this type of vaccination and indicates further studies to determine any direct tumor effect of this type of immunization.

IV. CURRENT LIMITS OF IMMUNOTHERAPY: IMMUNE ESCAPE

From the results of the studies described in this review, it is clear that an immune response can be generated in an individual against prostate cancer. This response is often not effective at eliminating the tumor, however. There are a myriad of reasons why an antitumor immune response may be ineffective (reviewed in Markiewicz and Gajewski, 1999), but one likely contributing factor is evasion of the immune response by the tumor. Tumor cell variants are known to emerge under immune pressure that lack or have mutations in molecules essential for recognition by immune cells. Loss or downregulation of class I MHC molecules has been found to be common in both prostate cancer cell lines and prostate cancer specimens (Bander *et al.,* 1997; Blades *et al.,* 1995; Sanda *et al.,* 1999). Because functional class I MHC molecules are required for prostate cancer cells to be targeted by CTL, this loss of class I MHC expression by prostate cancer cells is a major hurdle to T-cell-based therapies.

In addition to loss of expression of molecules critical to recognition by immune cells, prostate cancer cells can also negatively affect the immune system. Many tumor cells, including prostate cancer cells, secrete transforming growth factor β (TGF-β). TGF-β is immunosuppressive, inhibiting T cell, B cell, and NK proliferation and function. Plasma levels of TGF-β have been found to be elevated markedly in men with metastatic prostate cancer (Pirtskhalaishvili and Nelson, 2000; Stravodimos *et al.,* 2000). Such a high level of this immunosuppressive cytokine may inhibit the effectiveness of immunotherapy. Prostate cancer cells have also been shown to induce apoptosis of DC *in vitro* (Healy *et al.,* 1998; Herr, 1980; Salgaller *et al.,* 1998). If this

DC death occurs *in vivo,* it may disallow the generation of a tumor-specific T-cell response. Effective strategies to overcome such immunosuppression induced by prostate cancer cells will most likely need to be developed for immunotherapy against this disease to be successful.

V. CONCLUSIONS/FUTURE PERSPECTIVES

Several immunotherapeutic approaches have begun to be applied in the treatment of prostate cancer. Various approaches designed to stimulate a specific or nonspecific response to prostate cancer cells have been tested both in animal models and in the clinic. Although none have produced overwhelming clinical success, several have shown promise enough, especially in animal models, to warrant further exploration. It is likely that effective treatment will require these strategies, along with nonimmunotherapeutic strategies, to be used in multimodality approaches. With the development of the TRAMP model, a good animal model now exists in which thorough testing of possible therapies for prostate cancer can be done before testing in patients. In addition, although many prostate-specific antigens have been identified, there is a continued need to find additional prostate tumor-specific and tumor-associated antigens. With the current growth in genomics-based research, new markers are being identified at a rapid pace, providing an ever-increasing number of attractive candidates for immunotherapy.

For the generation of specific T-cell-mediated immunity, breaking immunological tolerance is a serious consideration because the known prostate cancer antigens are self-antigens. New technologies are currently being explored for their efficacy in overcoming T-cell tolerance, including antigen delivery, development of novel adjuvants, combinations of specific cytokines, and immunization with xenoantigens. In addition, evasion of tumor immunity is an issue that must be contended with. In those cases in which tumor cells lack MHC expression, mAb or NK-mediated therapy might be a preferable alternative to T-cell-based therapy. Treatments that improve APC function, which may be downregulated by the tumor, may also be critical in effective immunotherapy against this disease.

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BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
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EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

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Stanford University, Stanford, CA	A.B.	1976	Human Biology
University of Southern California, Los Angeles, CA	M.D.	1983	Medicine
LAC/USC Medical Center, Los Angeles, CA	Intern	1984	Surgery
LAC/USC Medical Center, Los Angeles, CA	Resident	1988	Urology
LAC/USC Medical Center, Los Angeles, CA	Fellow	1990	Urologic Oncology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. **DO NOT EXCEED TWO PAGES.**

1988-1990 Clinical Instructor, USC Medical School and Fellow, Urologic Oncology, LAC/USC Medical Center, Los Angeles, CA
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1976 Phi Beta Kappa, Stanford University
1982 Alpha Omega Alpha, USC
1983 Phi Kappa Phi, USC
1983 American Medical Womens Association, Glasgow Award for Academic Excellence
1988-1989 Clinical Oncology Fellow, American Cancer Society

PUBLICATIONS

Skinner EC, Lieskovsky G, Skinner DG. The technique of radical cystectomy. AUA Update Series Vol IX, No 7, 1990.

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